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17. MS-222 as an Anesthetic for Channel Catfish: Its Toxicity, Efficacy, and Muscle Residues

United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife

INVESTIGATIONS IN FISH CONTROL

Investigations in Fish Control, published by the Bureau of Sport Fisheries and Wildlife, include reports on the results of work at the Bureau's Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., and reports of other studies related to that work. Though each report is regarded as a separate publication, several may be issued under a single cover, for economy.

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15. Residues of MS-222 in Four Salmonids Following Anesthesia, by Charles R. Walker and Richard A. Schoettger. (Resource Publication 21.) 1967. 11p.
16. Annotated Bibliography on MS-222, by Richard A. Schoettger. (Resource Publication 22.) 1967. 15p.
17. MS-222 as an Anesthetic for Channel Catfish: Its Toxicity, Efficacy, and Muscle Residues, by Richard A. Schoettger, Charles R. Walker, Leif L. Marking, and Arnold M. Julin. (Resource Publication 33.) 1967. 14p.

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United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
Washington, D.C. • January 1967

FOREWORD

The Food and Drug Administration of the U.S. Department of Health, Education, and Welfare advised the Bureau of Sport Fisheries and Wildlife that certain drugs and chemicals used in the culture and management of game fishes must be cleared for continued use. We must demonstrate to the FDA that fish exposed to the compounds pose no danger, because of residues, to people who might eat them.

MS-222 is among the drugs mentioned because it is commonly used to anesthetize fish during marking or tagging, spawning by stripping, and transportation. The information necessary for clearance includes the toxicity of the drug to fish, its efficacy as an anesthetic, and its residues in fish. FDA also requires that fish of various sizes be represented in tests of the drug at three or more temperatures in waters of different qualities. Accordingly, we investigated the toxicity of MS-222 to eight species of game fish, its efficacy on four trouts, and its residues in the same trouts (emphasis was placed on residues in muscle because muscle is the principal tissue eaten by people).

MS-222 was synthesized by Maurice Sandoz (Sandoz, Ltd., Basle, Switzerland, and Hanover, N.J.) during his search about 45 years ago for a synthetic substitute for cocaine. It has been used as a local anesthetic in preparations for humans, but its general anesthetic activity against cold-blooded animals was recognized early. Within the past decade, its use as an anesthetic in fish culture and fish management has become widespread in the United States and abroad. For example, the millions of lake trout stocked in the upper Great Lakes in the past several years were first anesthetized with MS-222 and fin-clipped.

The considerable variety of common and chemical names applied to MS-222 in the literature causes some confusion. The names include:

MS-222.
MS-222 Sandoz.
M.S.-222 Sandoz.
TS-222.
TS-222 Sandoz.
Tricaine.
Tricaine - Sandoz.
Tricaine methanesulfonate.
Metacaine.
Metacaine methanesulphonate.
m-amino ethyl benzoate.
Ethyl m-aminobenzoate.
Methane sulfonic acid salt of meta-amino ethyl benzoate.
Methanesulphonate of meta-aminobenzoic acid.
Methanesulphonate meta-amino-benzoic acid ethyl ester.

Of these names, we used MS-222, tricaine methanesulfonate, and methanesulphonate of meta-aminobenzoic acid ethyl ester.

Robert E. Lennon, Director
Fish Control Laboratories

12. Toxicity of MS-222 to Selected Fishes

By Leif L. Marking

United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
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Resource Publication 18 • January 1967 • Washington, D.C.

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TOXICITY OF MS-222 TO SELECTED FISHES

By Leif L. Marking, Chemist,
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Abstract.--Toxicity of MS-222 to rainbow trout, brown trout, brook trout, lake trout, northern pike, bluegill, largemouth bass, and walleye of various sizes was determined in 15-, 30-, and 60-minute and 24-, 48-, and 96-hour static bioassays at selected temperatures. Twenty-four-hour LC_{50} values for the eight species ranged from 33.8 to 63.0 p.p.m. Exposures longer than 24 hours had little effect on toxicity. Small fish of a species were more sensitive to the drug than large ones, and trout were more sensitive at warmer temperatures. Safety indexes were calculated on the basis of the brief exposures.

Bové (1962) identified MS-222 as the methanesulphonate of meta-aminobenzoic acid ethyl ester. The compound is a fine, white crystalline powder with a molecular weight of 261.3 and a melting point of 145° to 150° C. It leaves only minimum traces of ash upon ignition and is free from chlorides, sulfates, alkaloids, and heavy metals. Less than 0.5 percent of its weight is lost upon heating to 103° C. It is soluble to 11 percent in water and forms a clear, colorless, acid, and relatively stable solution. He also reported that a 10-percent solution retained its potency during 3 days' storage, but decreased 5 percent in activity after 10 days. Exposure of a solution to light caused a change in color to yellow or brown, but its activity was not affected.

The molecular structure of MS-222, although not defined in literature, possibly conforms to figure 1. Since it is highly soluble (up to 22.5 percent in our laboratory) a proton bond probably exists between the amine group on the benzoate structure and the hydrogen on the methane sulfonante. In the ionization of the compound, the hydrogen probably splits off the sulfonate group, being attracted to the nitrogen of the amine group.

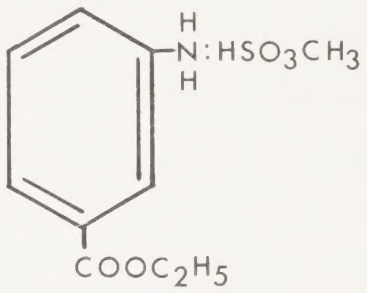

Figure 1.--Structure of MS-222.

MS-222 is an effective anesthetic for fish and other coldblooded animals (McFarland, 1959; Eisler and Backiel, 1960; Bové, 1962; Schoettger and Julin, 1966). Rothlin (1932) stated that the drug is three times less toxic to coldblooded animals than novocaine and ten times less toxic than cocaine.

There are reports, however, that under certain circumstances MS-222 is toxic to certain species or strains of fish. Marvin Smith (Regional Fishery Management Biologist at Atlanta, in a memorandum report to the Branch of Game Fish and Hatcheries, June 12, 1956) recommended that MS-222 be bioassayed for toxicity to rainbow trout under

various water qualities. Thompson (1959) observed a 95-percent mortality among cutthroat trout exposed to 50 p.p.m. of MS-222 for 5 hours. Others have noted that excessive exposures cause mortalities among fish (Nelson, 1953; Parkhurst and Smith, 1957; and Eisler and Backiel, 1960).

The purpose of this investigation was to define the concentrations of MS-222 which are toxic to eight species of freshwater, game fish of various sizes. The experimental conditions included different water qualities and durations of exposure at four temperatures. Safety indices for rainbow trout were established.

METHODS AND MATERIALS

Eight species of fish were obtained from various fish hatcheries (table 1). The three size groups included small fingerlings 1 to 3 inches long, an intermediate group of 3 to 5 inches, and a group 6 to 9 inches long. All fish were quarantined for 10 days and if judged acceptable, were then acclimated to the temperatures and conditions of the tests of MS-222.

The static bioassays of the anesthetic against 2-inch fish were conducted in 5-gallon glass jars (Lennon and Walker, 1964). At least 120 fish of this size were included in each test of 10 concentrations of drug. Ten fish were exposed to each concentration, and 20 served as controls. The tests against 4- and 9-inch fish were made in aerated, polyethylene tanks which contained 45 liters of anesthetic solution. Ten of the larger fish were exposed to each of 5 concentrations per test, and 10 served as controls.

TABLE 1.--Fishes used in toxicity tests of MS-222

	Source
Rainbow trout (*Salmo gairdneri*)	Manchester National Fish Hatchery, Iowa.
Brown trout (*Salmo trutta*)	Manchester National Fish Hatchery, Iowa.
Brook trout (*Salvelinus fontinalis*)	State Fish Hatchery at Osceola, Wis.
Lake trout (*Salvelinus namaycush*)	Jordan River National Fish Hatchery, Mich., and St. Croix Falls, Wis.
Northern pike (*Esox lucius*)	Garrison Dam National Fish Hatchery, N. Dakota and Yankton National Fish Hatchery, S. Dakota.
Bluegill (*Lepomis macrochirus*)	Lake Mills National Fish Hatchery, Wis.
Largemouth bass (*Micropterus salmoides*)	Genoa National Fish Hatchery, Wis.
Walleye (*Stizostedion vitreum*)	Garrison Dam National Fish Hatchery, N. Dakota.

Water temperatures were controlled by placing the bioassay vessels in water baths at 7°, 12°, 17°, and 22° C. Water hardnesses of 10, 42, and 176 p.p.m. were arranged by adding different amounts of the mixture of reconstituting salts to deionized water (table 2).

TABLE 2.--Water qualities obtained with various amounts of reconstituting salts in deionized water

Water quality	Salts in mg./l.				Total hardness (p.p.m.)	Total alkalinity (p.p.m.)	pH
	$NaHCO_3$	$CaSO_4$	$MgSO_4$	KCl			
Soft.....	12	7.5	7.5	.75	10	10	6.72
Standard..	48	30	30	3	42	30	6.75
Hard.....	192	120	120	12	176	116	7.70

Concentrated stock solutions of MS-222 were prepared daily in deionized water. Aliquots of the stock were added to bioassay vessels following the introduction of fish.

The responses of fish to MS-222 were recorded for several hours after exposure began and daily thereafter throughout the bioassay. It was possible during the tests to differentiate between dead and anesthetized fish. Dead specimens were removed immediately. Fish which remained in anesthesia throughout a test were placed in fresh water until they recovered. The times for recovery and survival were noted.

The data on survival and death of fish exposed to selected amounts of MS-222 were analyzed according to the method of Litchfield and Wilcoxon (1949) to define concentrations which produce 50-percent mortaility (LC_{50}). In addition, the variance and the 95-percent confidence intervals were determined.

RESULTS

MS-222 demonstrated a definite and consistent toxicity to the eight species of fish. Furthermore, the concentrations permitting survival or causing mortality can be predicted with a high degree of confidence.

Species and sizes of fish

Among the four trouts, the toxicity of MS-222 at a given temperature is dependent on variables such as species, size, and duration of exposure. Lake trout are the most sensitive and brook trout the least (table 3). Brown and rainbow trout are equal in their sensitivity and intermediate to lake and brook trout.

Larger fish of a species are usually more resistant to MS-222 than smaller fish. This is apparent with rainbow trout as the 24-hour LC_{50} increases from 39 p.p.m. for 2-inch fish to 52 p.p.m. for 9-inch fish. The trend is similar in 48- and 96-hour assays. The larger lake trout and brown trout also showed greater resistance than smaller fish at all periods of observation.

There was little difference in toxicity among brook trout of different sizes, but 6-inch specimens appeared to be more sensitive than 4-inch fish. It turned out, however, that the larger fish had become infected with furunculosis just prior to the bioassay, and quite possibly the disease lowered their resistance. A repeat trial with healthy brook trout was not possible.

Exposures beyond 24 hours do not have a pronounced effect on toxicity. In several instances the toxicity at 96 hours was identical to the 24-hour LC_{50}. Among the trouts, only 2-inch brown trout exhibited a significant difference, with a 20-percent drop in resistance between 24 and 96 hours.

Other species in the toxicity trials included northern pike, bluegill, largemouth bass, and walleye (table 4). Two-inch bluegill, largemouth bass, and walleye were only slightly less resistant than small brook trout. The larger specimens of bluegill and largemouth

TABLE 3.--Toxicity of MS-222 to salmonids at 12° C.

Species and lot	Average weight (grams)	Approximate length (inches)	At 24 hours		At 48 hours		At 96 hours	
			LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval
Rainbow trout:								
Lot 27	1.9	2	39.0	37.8-40.2	39.0	37.8-40.2	38.4	37.0-39.7
Lot 102	23.0	6	47.0	44.3-49.8	47.0	44.3-49.8	47.0	44.3-49.8
Lot 171	146.0	9	52.0	49.7-54.3	52.0	49.7-54.3	50.5	48.6-52.5
Brown trout:								
Lot 26	2.6	2	38.5	37.0-40.0	37.5	30.5-46.1	31.0	27.7-34.2
Lot 64	14.3	4	44.0	42.3-45.8	43.8	42.1-45.6	43.8	42.1-45.6
Lot 64	27.0	6	45.6	44.3-47.0	44.8	42.7-47.0	43.0	40.2-46.0
Brook trout:								
Lot 79	12.5	3	50.7	48.3-53.2	50.0	46.7-53.5	50.0	46.7-53.5
Lot 80	20.0	4	52.2	49.7-54.8	52.2	49.7-54.8	49.1	47.2-51.1
Lot 81	37.5	6	51.2	50.2-52.2	51.2	50.2-52.2	50.0	49.0-51.0
Lake trout:								
Lot 78	2.0	2	33.8	33.1-34.5	33.0	31.7-34.3	32.0	30.2-33.9
Lot 133	5.6	3	35.8	34.8-36.9	35.8	34.8-36.9	35.8	34.8-36.9
Lot 162	35.0	7	39.8	39.0-40.6	39.2	38.0-40.4	38.5	37.4-39.7

TABLE 4.--Toxicity of MS-222 to warm-water fish at 12° C.

Species and lot	Average weight (grams)	Approximate length (inches)	At 24 hours		At 48 hours		At 96 hours	
			LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval
Northern pike:								
Lot 35B	0.5	1	56.0	52.8-59.4	52.0	49.5-54.6	48.0	44.0-52.3
Lot 41	1.8	2						
Bluegill:								
Lot 112	1.3	2	45.7	44.8-46.6	45.7	44.8-46.6	45.7	44.8-46.6
Lot 120	2.8	3	46.9	46.0-47.8	46.9	46.0-47.8	46.9	46.0-47.8
Largemouth bass:								
Lot 57	0.5	1	42.0	40.8-43.3	42.0	40.8-43.3	39.4	36.5-42.6
Lot 70	5.2	4	47.0	44.3-49.8	46.5	44.7-48.4	46.5	44.7-48.4
Lot 91	63.0	7	61.5	55.9-67.6	57.9	55.1-60.8	57.9	55.1-60.8
Walleye:								
Lot 55	0.7	2	49.0	46.2-51.9	48.5	45.8-51.4	48.5	45.8-51.4

bass were more resistant than smaller ones. For example, the LC_{50} for 7-inch largemouth bass was 50 percent greater than for 1-inch bass. Also, all sizes of bluegill, largemouth bass, and walleye were affected very little by exposures longer than 24 hours.

The northern pike were of two sizes, but the data were insufficient for separate treatment and they were combined to facilitate statistical evaluation. Both sizes were healthy, but it is difficult to bioassay pike because they are voracious and turn to cannibalism unless other live food is available. Their possible hunger stress was demonstrated by decreased resistance at 96 hours.

Effects of temperature

The effects of temperature on the toxicity of MS-222 to rainbow trout which averaged 0.6 g. and 1.6 in. were evaluated (table 5). The trout were slightly more resistant at 7° than at 12° and 17° C. There was little difference between the LC_{50} values at 24 and 96 hours at a given temperature.

TABLE 5.--Toxicity of MS-222 to rainbow trout at selected temperatures

Temperature	At 24 hours		At 48 hours		At 96 hours	
	LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval
7°C.....	40.0	36.6-43.7	39.5	37.6-41.6	39.5	37.6-41.6
12°C.....	37.2	36.3-38.1	37.2	36.3-38.1	37.2	36.3-38.1
17°C.....	37.5	35.6-39.6	37.2	36.7-37.7	36.5	35.9-37.1

MS-222 was more toxic to small bluegills at 12° or 22° C. than at 17° (table 6). The differences, however, between LC_{50} values obtained at 3 temperatures in 24-, 48-, and 96-hour tests were not large. The fish tested at 22° were slightly smaller than the others, which may account for their greater sensitivity.

Effects of water quality

The effects of water hardness on the toxicity of MS-222 were established using rainbow trout which averaged 1.1 grams and 1.9 inches (table 7). The fish were from the same lot, and they were exposed concurrently to the anesthetic at three hardnesses. Results were consistent and accurate at 42 and 176 p.p.m. total hardness, but variable at 10 p.p.m. which necessitated several repeats for reliability. The inconsistent mortalities at lower concentrations indicated an erratic action of the drug in soft water. Partial mortality occurred at 33 and 34 p.p.m. in 24 hours, but no mortality occurred at 36 p.p.m. A previous, 24-hour test indicated considerably higher mortalities at 30 and 33 p.p.m. than at 38 p.p.m.

Recovery

The recovery of fish after 96-hour exposures was observed. At relatively low concentrations of MS-222, all fish recovered to some extent during the bioassay and completely during the recovery period immediately following the 96-hour exposure. At intermediate concentrations, which resulted in a partial kill during a 96-hour exposure, some of the survivors recovered partially previous to terminating the bioassay. Very few of the surviving fish died during the recovery period following exposure. The numbers of fish and the rates at which they recovered from anesthesia and survived exposures to the intermediate concentrations are listed in table 8.

The trout species recovered faster from partial-kill concentrations of MS-222 than the other fishes. Recovery was considered complete when specimens regained equilibrium and the ability to swim against a current.

TABLE 6.--Toxicity of MS-222 to bluegills at selected temperatures

Temperature	Lot No.	Average weight grams	Average length inches	At 24 hours		At 48 hours		At 96 hours	
				LC_{50}	95-percent confidence interval	LX_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval
12°C.....	131	1.1	1.7	43.0	41.7-44.3	43.0	41.7-44.3	43.0	41.7-44.3
17°C.....	131	1.1	1.7	45.0	44.1-45.9	44.9	43.6-46.2	44.0	42.7-45.3
22°C.....	152	0.8	1.6	39.8	39.0-40.6	39.8	39.0-40.6	39.8	39.0-40.6

TABLE 7.--Toxicity of MS-222 to rainbow trout in selected water qualities at 12° C.

Total hardness	Total alkalinity (p.p.m.)	pH	At 24 hours		At 48 hours		At 96 hours	
			LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval
10 p.p.m.	10	6.7	39.0	37.6-40.3	38.8	37.5-40.2	38.0	36.9-39.2
42 p.p.m.	30	6.8	40.0	39.1-40.8	39.0	38.1-40.0	39.0	38.1-40.0
176 p.p.m.	116	7.7	39.0	38.1-40.0	38.0	36.4-39.8	37.9	36.5-39.2

TABLE 8.--Recovery from anesthesia among fish exposed to concentrations of MS-222 causing partial kills within 96 hours at 12° C.

Species and length	Partial kill concentration (p.p.m.)	Number of fish		Minutes to recover in fresh water
		Surviving at 96 hours	Recovering after 96 hours	
Rainbow trout:				
2 inches	34-44	37	34	2- 5
6 inches	46-48	10	10	1- 2
9 inches	48-50	15	15	3- 4
Brown trout:				
2 inches	[illegible]	[illegible]	[illegible]	[illegible]
4 inches	[illegible]	[illegible]	[illegible]	[illegible]
6 inches	44-46	6	6	3- 13
Brook trout:				
3 inches	44-50	31	31	2- 9
4 inches	46-52	29	29	2- 20
6 inches	46-54	25	25	4- 15
Lake trout:				
2 inches	32-36	9	8	1- 2
3 inches	33-38	29	28	4- 12
7 inches	36-40	24	24	10- 30
Northern Pike:				
Bluegill:				
2 inches	47-54	14	13	2- 30
2 inches	44-46	18	18	10- 30
3 inches	45-47	29	29	2- 14
Largemouth bass:				
4 inches	44-56	10	10	5- 60
7 inches	46-56	45	45	5-120
Walleye:				
2 inches	40-50	34	32	60-180

Rainbow trout exposed to 34 to 50 p.p.m. for 96 hours recovered within 1 to 5 minutes when removed to fresh water. In contrast, walleye exposed to 40 to 50 p.p.m. for 96 hours required 60 to 180 minutes to recover. Of the 356 fish removed to and recovering in fresh water, only 8 subsequently died. Among the survivors, the 2-inch rainbow trout exposed to 34 to 44 p.p.m. of the drug for 96 hours and removsf to fresh water were observed for 7 days. There was no mortality; they fed readily and behaved similarly to controls.

Safety indexes

Rainbow trout averaging 1.4 grams and 2 inches were exposed to MS-222 in brief bioassays to determine safety indexes. A safety index refers to the margin between efficacy and mortality. It is expressed by the quotient of a lethal concentration and an effective concentration.

Safety indexes for rainbow trout in table 9 were derived from LC_{50} and EC_{50} values. The EC_{50} here relates to the concentration of drug which produces total loss of equilibrium in half of the specimens (Schoettger and Julin, 1966). The best results were obtained in 15-, 30-, and 60-minute exposures. Shorter exposures may be desirable from the point of view of field practice, but they give less accurate results. The fish do not die uniformly or quickly enough. Longer exposures of 1 to 6 hours were not meaningful because some fish recovered from anesthesia; others died at concentrations close to the effective concentration.

The maximum safety index (M.S.I.) is the quotient of the LC_1 and the EC_{99} . The values were extrapolated from the regressions used in determining the LC_{50} and EC_{50} . The M.S.I. is lower than the safety index and is biased in favor of greater safety.

TABLE 9.--Safety and maximum safety indexes of MS-222 against 2-inch rainbow trout in brief exposures at 12° C.

Exposure	Water hardness (p.p.m.)	Safety index			Maximum safety index		
		LC (p.p.m.)	EC_{50} (p.p.m.)	Index LC_{50}/EC_{50}	LC_1 (p.p.m.)	EC_{99} (p.p.m.)	Index LC_1/EC_{99}
15 minutes	42	64.6	32.0	2.0	54.0	38.2	1.4
30 minutes	42	56.8	31.8	1.8	49.5	38.5	1.3
60 minutes	42	55.5	29.2	1.9	46.0	36.8	1.3
15 minutes	10	64.0	34.0	1.9	50.0	47.5	1.1
30 minutes	10	58.0	31.5	1.8	46.5	45.0	1.0
60 minutes	10	54.2	31.2	1.7	44.0	40.4	1.1

The S.I. and M.S.I. derived for MS-222 against small rainbow trout demonstrate that the shorter exposures are safer for the fish than longer exposures (table 9). This was true in waters with hardnesses of 10 and 42 p.p.m. The data also show that the drug is not as safe in soft water as in harder water.

DISCUSSION

Toxicity of MS-222 to the eight species of fish depended on the concentration of drug and the duration of exposure. There was a straight-line relation between concentration and time of exposure within the initial 24 hours. Beyond 24 and up to 96 hours a given concentration showed little or no difference in toxicity. For example, the 15- and 30-minute and 24- and 96-hour LC_{50} values for 2-inch rainbow trout were 64.6, 55.5, 39.0, and 38.4 p.p.m. respectively. The 24- and 96-hour LC_{50} values for some species were identical, such as 47 p.p.m. for 6-inch rainbow trout.

The reasons for identical LC_{50} values at 24 and 96 hours may include a decrease of concentration by absorption and metabolism in the fish, some natural degradation of the drug in solution, and greater activity of the chemical on fish within the early hours of exposure.

It is noteworth that the LC_{50} values for the eight species did not differ greatly. The range of 24-hour LC_{50} values for the more susceptible lake trout to the more tolerant largemouth bass was 33.8 to 63.0 p.p.m.

Among the salmonids, brook trout were the more resistant to MS-222. Interestingly, Walker and Schoettger (1966), detected lower residues of the drug in brook trout than in rainbow, brown, and lake trout upon withdrawal from effective anesthesia. This may explain, in part, the higher resistance of the species to the anesthetic.

Water hardness did not influence the toxicity of MS-222 to rainbow trout, except that results at 10 p.p.m. were erratic. Parkhurst and Smith (1957) observed that rainbows react differently in various water supplies to the same concentrations of the drug. These inconsistencies suggest that the fish may not be exposed to MS-222 as safely in soft water as in hard water.

Sandoz Pharmaceuticals (in an undated leaflet "Toxicity of MS-222 to fish and frogs", 2 p.) stated that the therapeutic index (LC_1 / EC_{99}) of MS-222 for rainbow trout was 1.57. The LC_1, however, was defined in a 15-minute exposure of fish whereas the EC_{99} was defined in 3- to 4-minute exposures. Since the exposures for LC and EC differ, their index is not comparable to our safety indexes.

The S.I. (LC_{50}/EC_{50}) and the M.S.I. (LC_1/EC_{99}) displayed similar trends throughout the bioassays of MS-222 with one significant exception. There was little difference between the S.I. for rainbow trout in hard and soft water, but there was a significant difference between the M.S.I. The index was higher in the hard water. The slope of the regressions for LC and EC in soft water was less than in hard water, and the LC_1 was therefore smaller and the EC_{99} greater. These relations suggest that MS-222 is safer to rainbow trout in hard water than in soft. This was confirmed by Schoettger and Julin (1966) in their studies on the efficacy of the drug to rainbows in waters of various qualities. It is advisable to calculate both the S.I. and the

M.S.I. because one may suggest a possible hazard when the other doesn't.

The speed and degree of recovery of fish from anesthesia are important. Complete recovery from brief exposures is rapid, and it is proportional to the concentration of MS-222 and the duration of exposure according to Klontz (1964). This is not wholly the case in long exposures up to 96 hours. The concentrations of drug in long exposures did not hold all fish at a certain stage of narcosis for 96 hours. Some fish were recovering from anesthesia each day after the first 24 hours.

The trout recovered from anesthesia more rapidly than the other species. In fact, most fish recovered rapidly, and the extended recovery times for some species reflect the very slow recoveries of a few individuals.

Sublethal exposures to MS-222 did not seem to harm fish. Rainbow trout which survived partial-kill concentrations were observed for 7 days. They fed and behaved as well as controls.

CONCLUSIONS

MS-222 demonstrated a definite and consistent toxicity to eight species of fish in 24- to 96-hour bioassays. The range of 24-hour LC_{50} values for the more susceptible lake trout to the more tolerant largemouth bass was 33.8 to 63.0 p.p.m. The toxicity was not much greater in exposures of more than 24 hours.

Larger specimens of a species are usually more resistant to MS-222 than small ones.

The trout were slightly more resistant to the anesthetic at colder temperatures. Bluegills were more resistant at 17° than at 12° to 22° C.

Toxicity of MS-222 to rainbow trout was influenced very little by water hardness, but the fish reacted inconsistently in very soft water.

Among the survivors in partial-kill concentrations of MS-222 in 96-hour exposures, the trout recovered from anesthesia more rapidly than other species.

Safety indexes based on the quotient of lethal and effective concentrations can be used to distinguish the margin between toxicity and efficacy.

SUMMARY

Toxicity of MS-222 to rainbow trout, brown trout, brook trout, lake trout, northern pike, bluegill, largemouth bass, and walleye of various sizes was determined in 15-, 30-, and 60-minute and 24-, 48- and 96-hour bioassays at selected temperatures. The 24-hour LC_{50} values for the more sensitive lake trout and more tolerant largemouth bass ranged from 33.8 to 63.0 p.p.m. and mortalities were not much greater in exposures which exceeded 24 hours.

Larger fish of a species are more resistant to the drug than small ones. Also, the trout were more tolerant at colder temperatures. Bluegills, on the other hand, were more tolerant at 17° than at 12° and 22° C.

The drug was tested against rainbow trout in waters with hardnesses of 10, 42, and 176 p.p.m. The fish reacted inconsistently in the softer water.

The recovery from anesthesia was evaluated in fish which survived partial-kill concentrations of MS-222 in 96-hour exposures. The trout recovered more rapidly than the other species, and posttreatment survival was good.

Safety indexes (LC_{50}/EC_{50}) and maximum safety indexes (LC_1/EC_{99}) were calculated from data obtained in 15-, 30-, and 60-minute exposures of rainbow trout to MS-222. They can be used to estimate the margin of safety between toxicity and efficacy. In one instance, the maximum safety index more clearly demonstrated the possible hazard of exposing small rainbow trout to the drug in soft water than did the safety index.

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13. Efficacy of MS-222 as an Anesthetic on Four Salmonids

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EFFICACY OF MS-222 AS AN ANESTHETIC ON FOUR SALMONIDS

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Abstract.--MS-222 was tested for its efficacy as an anesthetic for rainbow trout, brown trout, brook trout, and lake trout. Eighty to 135 p.p.m. of MS-222 anesthetized fish within 3 minutes at 7° to 17° C. The fish could be exposed for a total time of 4 to 12 minutes. Fifty to 60 p.p.m. induced a moderate rate of anesthesia which could be maintained for approximately 30 minutes. Sedation was produced within 15 minutes and maintaine for 5 to 6 hours at 15 to 30 p.p.m. The efficacy of sedating concentrations appeared to decrease with time at 17° C. Lake trout required larger doses than the other salmonids for complete anesthesia but tolerated only short exposures. There was no relation between size of fish and efficacy of MS-222. Smaller fish occasionally had shorter exposure times. The drug was equally efficaceous at pH values of 5.0, 7.0, and 8.5. Anesthetic solutions with a total hardness of 10 p.p.m. were less effective in anesthetizing rainbow trout than those containing 35 and 180 p.p.m. Individuals which were anesthetized in soft water recovered sooner.

MS-222 (tricaine methanesulfonate) was discovered by Maurice Sandoz during investigations to develop a synthetic substitute for cocaine. It was classified as a local anesthetic for use in human medicine. During the late 1920's, the drug also came into use as an anesthetic for poikilotherms including fish, salamanders and frogs (Bové, 1962).

The potential usefulness of MS-222 was not recognized widely in fisheries until Wood (1956) and Ball and Cowen (1959) reported on the carcinogenic properties of urethane, then a commonly used anesthetic for fish. An editorial comment accompanying Wood's report indicated that MS-222 might be a suitable substitute for urethane. Since then, the anesthetization of fish with MS-222 has become routine to facilitate the handling of both marine and freshwater species. The toxicity of the drug to fish was determined by Marking (1966). Walker and Schoettger (1966) measured its residues in various tissues of salmonids.

General guidelines for effective and safe use of MS-222 in fisheries are frequently misleading or lacking. The concentrations employed in fish culture, fisheries management and research were reviewed by Bové (1962), the manufacturer[1], Eisler and Backiel (1960), and Schoettger (1966). They differ widely depending on factors such as species and temperature. Unfortunately, potentially lethal concentrations may be required to achieve desired rates or depths of anesthesia. Thus, the durations of exposure are critical. If many fish are anesthetized at one time, some may become over exposed and die. Concentrations for selected rates of anesthesia, exposure times tolerated by the fish and factors affecting the drug's efficacy therefore

[1] Sandoz Pharmaceuticals: (No date) M.S. 222-Sandoz, the anesthetic of choice in work with cold-blooded animals. Sandoz Pharmaceuticals, Hanover, N.J., Technical Bulletin. 10 p.

required definition under controlled conditions.

Because of the widespread use of MS-222 in the culture and management of salmonids, we selected four representatives of the group for this study. The influences of temperature, water hardness, pH, repeated exposure and size of fish on the action of the anesthetic were evaluated.

METHODS AND MATERIALS

The efficacy of MS-222 was tested against rainbow trout, brown trout, brook trout, and lake trout of 2 to 6 inches and 7 to 12 inches (table 1). The test fish were held in well water at 12° C. and starved for two days prior to their placement in the anesthetic solutions. Twenty-four hours before an experiment, the fish were temperature conditioned in reconstituted water which was prepared according to methods described by Lennon and Walker (1964). Fish less than 4 inches long were tested in 15 liters of reconstituted water. Larger individuals were tested in 45 liters. Since preliminary tests failed to show that variable loading had any effect on efficacy, the loading levels were approximately 10 g./l. for the smaller fish, and up to 40 g./l. for the larger fish.

TABLE 1.--Trouts used in tests of MS-222

	Source
Rainbow trout (*Salmo gairdneri*).....	Manchester National Fish Hatchery, Manchester, Iowa.
Brown trout (*Salmo trutta*).....	Manchester National Fish Hatchery, Manchester, Iowa, and State Fish Hatchery at Lanesboro, Minn.
Brook trout (*Salvelinus fontinalis*).	State Fish Hatcheries at Osceola, Wis., St. Croix Falls, Wis., and Lanesboro, Minn.
Lake trout (*Salvelinus namaycush*)...	Jordan River National Fish Hatchery, Charlevoix, Mich., and State Fish Hatchery at St. Croix Falls, Wis.

Initial trials with MS-222 demonstrated the need to establish: (1) general behavioral responses of fish to the anesthetic, and (2) criteria to evaluate effective concentrations.

The behavioral responses of fish treated with MS-222 are shown in table 2. Similar responses were reported by McFarland (1959 and 1960) and Klontz (1964). Recognition of these stages of anesthesia is essential to the selection of dosage rates, and to reduce the risk of excessive exposures.

TABLE 2.--General behaviorial response of fish which characterize progressive levels of anesthesia

Sedation.....	Decreased reactivity to visual and vibrational stimuli; opercular and locomotor activity reduced slightly; darker in color.
Partial loss of equilibrium..	Loss of equilibrium in water current; increased opercular rate; swimming ability disrupted.
Total loss of equilibrium:	
Stage 1.....	Usually turn over; swimming ability persists; opercular rate rapid; react to vibrational stimuli.
Stage 2.....	Locomotion ceases; fin movement may continue; tactile response only to pressure on caudel fin or peduncle; opercular rate slowed.
Loss of reflex activity.....	Failure to respond to external stimuli, particularly pressure on caudal fin or peduncle; opercular rate slow and erratic.
Medullary collapse.....	Opercular activity ceases.

The criteria established to determine the efficacy of MS-222, though somewhat arbitrary, were based on general considerations reported in the literature (table 3). It is difficult to predict concentrations of MS-222 which satisfy all requirements of an anesthetic; however, the levels associated with these criteria provide reference points which are adaptable to the specific needs for an anesthetic. The periods that fish tolerated exposure to MS-222 were determined by entry of the first and the last individual into medullary

Table 3.--Criteria for testing anesthetics

Use	Desired effect	Criteria for effective concentration
Handling: Fin clipping, stripping, etc.....	A. Rapid immobility Rapid recovery. Brief immersion time.	A. Concentration producing loss of reflex in all fish within 3 minutes. Total immersion time dependent on the onset of medullary collapse.
	B. Moderately rapid and sustained anesthesia.	B. Concentration producing total loss of equilibrium, stage II, in all fish within 15 minutes. Fish recover after 15 to 30 minutes of immersion.
Transportation of fish.....	Reduced activity. Reduced oxygen consumption. Dispersal of fish in containers. Rapid recovery.	Concentration producing sedation within minutes and maintaining it for 5 to 6 hours.

collapse. The time of response is useful in estimating the numbers of fish which can be handled safely in a batch. In actual practice the more susceptible fish respond first to the anesthetic and are removed.

A series of concentrations of MS-222 were tested at a temperature of 12° C. and at pH 7.0. The levels giving reasonable anesthetization, holding and recovery times, and which produced minimum mortality were considered for further trials at 7°, 12° and 17° C., and at pH 5.0, 7.0, and 8.5. Temperature control was maintained by placing the test containers in water baths. The pH of some solutions was varied by the addition of sodium bicarbonate, or potassium acid phthalate and sodium hydroxide. The oxygen concentrations of the solutions were maintained close to saturation by aeration before, or during the experiments.

The metabolic rates of fish sedated with MS-222 were compared with those of unanesthetized fish. The studies were conducted in open and closed systems, and were designed to estimate the usefulness of the drug for suppressing the metabolism of fish in tanks, and in plastic bags.

Lake trout averaging 4 inches in length and 6.3 grams in weight were used in the open-system tests. Five individuals were placed in each of sixteen 1-gallon jars, each containing 2.5 liters of reconstituted water and the volume adjusted slightly to achieve a constant loading of 15 g./l. Eight test solutions at 12° C. contained sufficient MS-222 to produce sedation (20 p.p.m.), and were saturated with oxyben. At intervals, one container of fish from each experimental and control group was selected randomly and the oxygen was measured by the modified Winkler method. The tests continued until the fish showed signs of distress because of low oxygen.

In the closed-system trials, one or two 6-inch rainbow trout were weighed and sealed in 1-gallon jars that were completely filled with reconstituted water at 12° C. The solutions were saturated with oxygen at the beginning of the tests. The fish were sedated with 20 to 30 p.p.m. of MS-222 and the quantities of oxygen consumed by them were compared to controls after 30 to 60 minutes. The values were corrected for the loading level in each jar.

The influence of water hardness on the efficacy of MS-222 was measured with 4-inch and 9-inch rainbow trout. The hardnesses of the various solutions were altered by adding different amounts of the mixed reconstituting salts. Preliminary tests were conducted in solutions of MS-222 which had hardnesses ranging from 17 to 350 p.p.m. as calcium carbonate. In subsequent trials a concentration of 100 p.p.m. of the drug was tested for efficacy in solutions with hardnesses of 10 and 180 p.p.m. These contained 5 and 75 p.p.m. of calcium ion, 1.5 and 7.0 p.p.m. of chloride ion, 9.5 and 116 p.p.m. total alkalinity, and had pH's of 7.14 and 8.20 respectively. They will later be referred to as relatively soft and hard waters. The trout were acclimated in both types of water for 24 to 48 hours preceeding the experiments and were placed in well water for recovery.

The effect of repeated exposure of fish to MS-222 was tested by anesthetizing 12-inch rainbow trout and 6-inch lake trout daily in a concentration of 100 p.p.m. The time preceeding the onset of medullary collapse, recovery time and survial were used as indices of sensitivity. The trials with rainbow trout continued for 21 days and were conducted in reconstituted water at 12° C. The fish were placed in well water for recovery. In similar experiments lake trout received 12 treatments. Their sensitivity to the anesthetic was compared periodically with untreated individuals. The fish were anesthetized and recovered in well water. Both species were tested in groups of 10 and fed their normal ration throughout the trials.

RESULTS AND DISCUSSION

The efficacy of MS-222 for inducing rapid and moderately rapid anesthesia and sedation in two size groups of salmonids was tested at 7° to 17° C. and pH 5.0 to 8.5. The efficacy of the drug appeared unaltered by pH and these data were grouped, without differentiation, with those for size of fish and temperature.

Rapid anesthesia

Rainbow trout were anesthetized to loss of reflex within 3 minutes in solutions containing 100 p.p.m. of MS-222 (table 4). Fish in both size ranges were equally sensitive to the drug and in contrast to other salmonids, there appeared to be less effect of temperature on efficacy. The fish withstood exposure for approximately 5.5 to 12 minutes. The durations were somewhat shorter at higher temperatures and with smaller fish.

A concentration of 80 p.p.m. was 100-percent effective on brown trout at 17°, however, the concentration was raised to 100 p.p.m. at 7° and 12° C. to achieve efficacies of 86.6 to 100 percent (table 4). Though a weaker solution was used at 17°, the fish reached medullary collapse and recovered sooner than at 7° and 12°. This suggests a greater metabolism of MS-222 at higher temperatures.

Brook trout and lake trout were effectively anesthetized by 100 p.p.m. of MS-222 at 17°

TABLE 4.--Concentrations of MS-222 producing rapid anesthesia in four salmonids at three temperatures.

Species and concentration	Temperature (C.)	Size of fish inches	Fish in loss of--				Mean range of exposure times (minutes)		Safe exposure index[1]	Recovery	
			Equilibrium, stage 11, with 2 minutes		Reflex within 3 minutes		First fish	Last fish		Mean time range minutes	Survival (percent)
			Number	Percent	Number	Percent					
Rainbow trout:											
100 p.p.m.	7°	2-6	60/60	100.0	60/60	100.0	6.6	8.1	2.2	4.0-11.0	98.4
Do.	7°	7-12	20/20	100.0	20/20	100.0	6.8	11.4	2.3	5.1-11.8	100.0
Do.	12°	2-6	173/173	100.0	170/173	98.3	5.5	7.0	1.8	2.8- 8.2	93.1
Do.	12°	7-12	95/95	100.0	95/95	100.0	8.0	11.9	2.7	4.2- 8.6	100.0
Do.	17°	7-12	20/20	100.0	20/20	100.0	5.5	6.9	1.8	4.3- 9.0	93.3
Brown trout:											
80 p.p.m.	12°	2-6	20/20	100.0	13/20	65.0	6.9	13.3	2.3	3.3- 6.6	100.0
Do.	17°	2-6	5/5	100.0	5/5	100.0	5.3	7.3	1.8	6.0- 6.3	40.0
Do.	17°	7-12	20/20	100.0	20/20	100.0	4.6	7.0	1.5	3.9- 8.2	86.6
90 p.p.m.	7°	2-6	13/15	86.6	9/15	60.0	9.4	10.4	3.1	5.8-10.1	100.0
Do.	12°	2-6	40/40	100.0	39/40	97.5	5.9	8.3	2.0	3.8- 7.6	100.0
Do.	17°	7-12	8/8	100.0	8/8	100.0	4.5	6.1	1.5	4.8-17.0	75.0
100 p.p.m.	7°	2-6	30/30	100.0	26/30	86.6	7.9	9.4	2.6	6.4-18.5	100.0
Do.	7°	7-12	34/35	97.1	32/35	91.4	9.5	12.1	3.2	7.3-11.0	96.6
Do.	12°	2-6	60/60	100.0	60/60	100.0	6.1	8.1	2.0	4.1- 9.6	100.0
Do.	12°	7-12	55/55	100.0	53/55	96.3	7.9	11.0	2.6	4.9-13.3	98.1
Brook trout:											
100 p.p.m.	12°	2-6	70/70	100.0	43/60	71.6	5.0	--	1.7	2.0- 6.4	100.0
[illegible]	17°	7-12	20/20	100.0	20/20	100.0	4.2	5.4	1.4	5.1- 8.9	93.3
110 p.p.m.	12°	7-12	30/30	100.0	30/30	100.0	6.5	8.7	2.2	4.4- 7.6	100.0
120 p.p.m.	7°	7-12	24/25	96.0	24/25	96.0	8.8	11.4	2.9	4.1- 7.7	100.0
Lake trout:											
100 p.p.m.	7°	2-6	38/120	31.6	10/120	8.3	6.9	8.5	2.3	5.5-19.2	85.8
Do.	12°	2-6	101/120	84.1	71/120	59.2	4.7	5.4	1.6	4.1-15.8	91.7
Do.	17°	7-12	25/25	100.0	25/25	100.0	3.9	4.7	1.3	3.4- 5.3	100.0
110 p.p.m.	12°	7-12	30/30	100.0	29/30	96.7	5.0	6.3	1.7	3.5- 9.1	100.0
135 p.p.m.	7°	7-12	18/25	72.0	20/25	80.0	5.6	6.5	1.9	4.4- 7.1	100.0

[1] Index obtained by dividing the time for the first fish to reach medullary collapse by the time (3 minutes) for fish to reach loss of reflex.

and by 110 p.p.m. at 12° (table 4). The dosage was raised to 120 p.p.m. for brook trout, and 135 p.p.m. for lake trout at 7°. The concentrations for lake trout are similar to those used at a national fish hatchery[2] in Michigan.

In general, the chars were more resistant to MS-222 at 7° and 12° than rainbow trout or brown trout. This may indicate a lower, and possibly narrower, range of optimum temperatures for the former species. The exposures for brook trout ranged from 4.2 minutes at 17° to 11.4 minutes at 7°. The values for lake trout, at the same temperatures, were 3.9 and 6.5, and they reflect a greater sensitivity than the other salmonids to prolonged anesthetization. Also, the lake trout died quickly when they were not removed from the anesthetic soon after the cessation of respiration. This was not as critical with the other species and suggests that the respiratory and cardiac centers of lake trout are inhibited at a similar rate by the drug.

[2] Personal communication from George Drake, Hatchery Manager, Pendills Creek National Fish Hatchery, Brimley, Mich.

The progress of anesthesia in the various salmonids slowed, and exposure times lengthened with decreasing temperature. Although lower dosages were used at 17° C., with the exception of rainbow trout, it was necessary to remove the fish from the anesthetic solution sooner than at 7°. The reduced efficacy at relatively low temperatures is in accord with the results of Meister and Ritzi (1958). We concluded that rapid anesthesia is more safely induced and maintained at lower temperatures.

The drug appeared to be similarily effective on both size ranges of rainbow trout and brown trout; however, larger fish withstood longer anesthesia. Brook trout and lake trout of smaller sizes were not tested at the concentrations used for larger specimens. The trouts usually recovered from deep anesthesia within 3 to 15 minutes. There were no consistent relations between recovery time and temperature, size of fish, or total exposure.

MS-222 is capable of inducing rapid anesthesia which makes it useful in spawning, marking, and measuring fish, and in immobilizing specimens for various physiological investigations (Nelson, 1953; Butler, 1957; Parkhurst and Smith, 1957; Schiffman and Fromm, 1959; Crawford and Hulsey, 1963; Black and Connor, 1964). Its relatively high narcotic potency to fish is related to its molecular weight (McFarland, 1959).

The rate of anesthetization with MS-222 can be controlled by the dosage. According to the literature, rapid results have been achieved with concentrations ranging from approximately 80 to 1,000 p.p.m. The action of the drug is reversible at these concentrations provided exposure time is manipulated to minimize mortality. We observed that anesthetized trout always recovered when they were transferred to fresh water before cessation of respiratory activity. Rodman (1963) found that anesthesia of white sturgeon was not reversible when the fish were exposed to a concentration of 1:40,000 for 30 to 48 hours. Marking (1966) maintained rainbow trout under anesthesia with MS-222 for 96 hours. They recovered quickly in fresh water and appeared to be healthy seven days later.

Our results, and those of other investigators, demonstrate that relatively high concentrations of MS-222 are effective and safe for the rapid anesthetization of trout. The dependence of survival on exposure time suggests than an index for the safe exposure of fish to MS-222 can be obtained from a quotient of the time for medullary collapse and that for anesthesia. Indexes for the various species and sizes of fish were calculated for temperatures of 7°, 12°, and 17° C. (table 4). They are biased in favor of greater safety. High indexes may be calculated from times for shallower anesthesia and longer exposure. Usually, the indexes were greater than 1.5. The values of 12° and 17° were relatively lower than those at 7° and indicated a greater hazard in anesthetizing fish with MS-222 at higher temperatures. Brook trout and lake trout, for example, had respective indices of 1.4 and 1.3 at 17°, whereas the values were 2.9 and 1.9 at 7° C.

Effects of water hardness.--In preliminary studies, we found that the water harnesses from 17 to 350 p.p.m. had little influence on the anesthetic properties of MS-222 against rainbow trout.

Phillips et al. (1955) established the importance of the calcium ion in the metabolism and osmoregulation of brook trout. Calcium concentrations of 5 p.p.m. increased metabolism, presumably in active opposition to the osmotic inflow of water. We anticipated that solutions with a hardness of 10 p.p.m. and a calcium ion level of 5 p.p.m. might introduce an osmotic stress which would alter the anesthetic action of MS-222 against rainbow trout. The results of trials in solutions with hardnesses of 10 p.p.m. and 180 p.p.m. are shown in table 5. In soft water, the times to

TABLE 5.--Effect of water hardness on the efficacy of 100 p.p.m. of MS-222 against 9-inch rainbow trout at 12° C.

	Mean time in minutes to--					
	Loss of reflex in water with hardness of--		Medullary collapse in water with hardness of--		Recovery in water with hardness of--	
	10 p.p.m.	180 p.p.m.	10 p.p.m.	180 p.p.m.	10 p.p.m.	180 p.p.m.
Six tests (80 fish).....	7.98	2.43	16.30	8.10	4.68	6.6
Standard deviation.....	1.98	0.29	3.61	1.94	1.57	2.22
Differences between means	5.55		8.70		1.99	
Calculated "t".....	17.56*		13.41*		4.98*	

*Significant at the 0.01 level.

reach complete anesthesia and durations of exposure were double those for hard water, and recovery times were shorter. The mean differences between soft and hard water were statistically significant at the 0.01 level. Onkst et al. (1957) observed that induction of anesthesia in guppies with pentobarbital was inhibited when the fish were acclimated and tested in a calcium dificient medium. Marking (1966) observed that higher concentrations of MS-222 were required to anesthetize rainbow trout in soft water.

The results in hard water agree closely with those in table 4 which were determined in waters with a hardness of 35 p.p.m. The data in tables 4 and 5 indicate that there is a threshold of ions below which the efficacy of MS-222 is partially inhibited. Ions other than calcium may also contribute to this effect.

The reduced effectiveness of MS-222 in soft water is not due to lower uptake of the drug. Greater amounts of MS-222 were found in the muscle tissues of fish anesthetized in soft water than in those treated in hard water (Walker and Schoettger, 1966). The longer exposures of trout in soft water, and increases in their metabolism to maintain osmotic equilibrium, may account for the higher levels of MS-222 in these fish.

We observed that the rates of anesthetization of fish to total loss of equilibrium, stage 1, were similar in both soft and hard water. The later stages of anesthesia, however, occurred slowly in soft water. Using McFarland's (1959) suggested classification of anesthesia in fishes, there appeared to be no change in the depressive action of MS-222 on the telencephalon, sensory areas of the tectum and tegmentum, and midbrain and thalamus of fish treated in soft water. Depressions of the motor nuciei in the medulla, the spinal reflex arcs, and of respiratory and cardiac centers are responsible for deeper anesthesia. It is possible that in these areas the activity of the anesthetic was antagonized by osmotic disturbances of the potassium-calcium balance in the nerve cells. Also, considering the results of Phillips et al. (1955) and Walker and Schoettger (1966), the rate of absorption and deactivation of MS-222 may have increased along with higher metabolic rates of fish in soft water.

Phillips et al. (1957) showed that the narcotization of brook trout with MS-222 increased their absorption of radioactive cobalt. They suggested that there may be an adjustment of the osmotic processes of narcotized fish. According to Quastel (1963) anesthetics have a twofold mechanism of action on the respiration and metabolism of brain tissue: (1) effects on the cationic equilibrium of the cell membrane, and (2) the anesthetic may affect the oxidation of diphosphopyridine nucleotide within the cell. The first mechanism may occur without the second. Changes in ionic balance such as an increase in cellular potassium or a decrease in calcium stimulate respiration of the nerve cell. An anesthetic may deactivate carriers at the cell membrane which are responsible for cation movements, and have an effect similar to that of a respiratory inhibitor acting on the mitochondria. It is not known whether the action of MS-222 on the central nervous system of fish is in accord with this scheme for other anesthetics.

Effects of repeated anesthetization.--The daily anesthetization of 10 rainbow trout over 21 days, and 10 lake trout over 12 days with 100 p.p.m. of MS-222 produced no progressive increase, or decrease in their tolerance to the drug. The tolerances of lake trout were compared periodically with those of previously untreated individuals. The repeatedly exposed fish withstood anesthesia for approximately 1.0 to 1.5 minutes longer than the control group. The higher tolerance apparently originated after the first treatment. Recovery time and survival were not affected by repeated exposure. Klontz (1964) reported a slight increase in tolerance of fish anesthetized repeatedly with MS-222 which was compensated by increasing the concentration.

Moderately rapid anesthesia

A moderate rate of anesthesia is useful in situations where fish must be handled over a longer time, but where rapid immobilization is not essential. For example, MS-222 is used to anesthetize fish during surgery and hematological studies (Robertson, 1958; Smith and Bell, 1964).

We determined that concentrations of 50 to 60 p.p.m. anesthetized the four salmonids to total loss of equilibrium, stage 2, within 15 minutes (table 6). The fish usually could remain in the anesthetic for 30 minutes.

A concentration of 60 p.p.m. was effective in anesthetizing rainbow trout within 15 minutes, but attempts to maintain them under anesthesia for 30 minutes resulted in mortalities of approximately 8 to 25 percent. More individuals died at 12° than at 7° C. The anesthetic was almost as effective at 50 to 55 p.p.m., and mortality was less.

MS-222 was effective on brown trout at 50 p.p.m., and only 1 fish of 170 failed to recover (table 6).

The chars were relatively less sensitive to MS-222 than the other fish and a concentration of 60 p.p.m. was required to induce anesthesia within 15 minutes. The data indicate that there was a slight reduction in efficacy of the drug at 12° C., which suggests some deterioration, or increased metabolism, of MS-222 in higher temperatures. Although lake trout appeared to be resistant, some did not tolerate exposures to 60 p.p.m. longer than 15 minutes. A concentration of 50 p.p.m. lengthened the immersion time, but reduced the initial speed of anesthetization.

All of the brook trout recovered from anesthesia, but mortalities of lake trout ranged from 15 to 35 percent and were greater at 12° than at 7° C. The mortalities were probably related to our inability to pinpoint the onset of medullary collapse. The duration of the loss of reflex in lake trout is very brief.

The recovery of fish in these tests usually occurred within 1 to 15 minutes.

Sedation

MS-222 can be used to produce sedation in fish. Solutions containing 20 p.p.m. of the drug were, with several exceptions, 80- to 100-percent effective in sedating rainbow trout (table 7). Brown trout were sedated by 15 p.p.m. Thirty p.p.m. were required for larger rainbow trout at 17° and for brook trout at 7° C. The trout usually recovered within 1 minute after they were transferred to fresh water. Concentrations similar to these have been used to sedate white sturgeon, sockeye salmon, cutthroat trout, rainbow trout, and bluegill (Rodman, 1963; Meehan and Revet, 1962; Thompson, 1959; Dollar, 1963; Gebhard, 1965; Webb, 1958). Lamarque (1964) recommended dosages of 20 to 100 p.p.m., depending on temperature, for the preliminary anesthesia of fish before they are placed in plastic bags for shipment. He suggested 10 p.p.m. for tranquilization of fish in hauling tanks.

McFarland (1959) stated that pH may influence the absorption of anesthetics into the gills of fish. Our results, however, showed no

TABLE 6.--Concentrations of MS-222 producing moderately rapid anesthesia in fish to total loss of equilibrium, stage 2, within 15 minutes

Species and concentration	Temperature (C.)	Size of fish (inches)	Fish in anesthesia		Exposure time (minutes)	Recovery	
			Number	Percent		Mean time range (minutes)	Survival (percent)
Rainbow trout:							
50 p.p.m.	7°	2-6	43/45	95.5	15->30	1.40- 4.00	100.0
Do.	12°	2-6	182/210	86.7	>30	1.59-17.54	98.9
55 p.p.m.	12°	2-6	20/20	100.0	30	2.75-11.25	100.0
60 p.p.m.	7°	2-6	134/135	99.2	15->30	3.40- 9.20	91.8
Do.	12°	2-6	85/85	100.0	15- 30	2.20-16.60	75.2
Brown trout:							
50 p.p.m.	7°	2-6	30/30	100.0	30	4.40- 6.80	100.0
[illegible]	7°	7-12	10/10	100.0	30	6.20-52.00	90.0
[illegible]	12°	2-6	130/130	100.0	30	2.30- 4.60	100.0
Brook trout:							
60 p.p.m.	7°	2-6	40/40	100.0	20- 30	3.20- 8.00	100.0
Do.	12°	2-6	50/60	83.3	30	3.20- 5.70	100.0
Lake trout:							
40 p.p.m.	12°	2-6	0/10	0.0	30	1.00- 1.50	100.0
50 p.p.m.	7°	2-6	72/120	60.0	30	3.41-13.57	83.3
Do.	12°	2-6	47/140	33.5	15- 30	2.23-15.23	65.8
60 p.p.m.	7°	2-6	133/140	95.0	15- 30	3.70-13.10	87.5
[illegible]	12°	2-6	84/120	74.0	15- 30	1.00-21.80	68.0

TABLE 7.--Concentrations of MS-222 producing sedation in four salmonids

Species and concentration	Temperature (C.)	Size range (inches)	Fish in sedation at--				Behavior[1] of fish not in sedation at--	
			15 minutes		5-6 hours			
			Number	Percent	Number	Percent	15 minutes	5-6 hours
Rainbow trout:								
20 p.p.m.	7°	2-6	132/135	97.8	129/135	95.6	>	>
Do.	7°	7-12	80/120	66.6	78/120	65.0	>	>
Do.	12°	2-6	175/177	98.8	177/177	100.0	>	
Do.	12°	7-12	81/90	90.0	84/90	93.3	>	>
Do.	17°	7-12	32/40	80.0	22/40	55.0	<	<
30 p.p.m.	12°	7-12	20/20	100.0	16/20	80.0		>
Do.	17°	7-12	14/20	70.0	16/20	80.0	>	>
Brown trout:								
15 p.p.m.	7°	2-6	20/35	57.1	33/35	94.3	>	>
Do.	7°	7-12	45/50	90.0	49/50	98.0	>	>
Do.	12°	2-6	28/30	93.3	30/30	100.0	>	
Do.	17°	7-12	20/20	100.0	20/20	100.0		
20 p.p.m.	7°	2-6	9/36	25.0	23/36	63.9	>	>
Do.	12°	2-6	21/72	29.2	70/72	97.2	>	>
Do.	12°	7-12	17/20	85.0	20/20	100.0	>	
Brook trout:								
20 p.p.m.	7°	2-6	10/10	100.0	5/10	50.0		<
Do.	12°	7-12	20/20	100.0	20/20	100.0		
Do.	17°	7-12	20/20	100.0	20/20	100.0		
[illegible] p.p.m.	7°	2-6	57/60	95.0	50/60	83.3	>	>
Do.	7°	7-12	20/20	100.0	19/20	95.0		>
Do.	12°	2-6	29/30	96.7	24/30	80.0	>	>
Do.	12°	7-12	9/20	45.0	20/20	100.0	>	
Do.	17°	7-12	12/20	60.0	15/20	75.0	>	< >
Lake trout:								
20 p.p.m.	7°	2-6	179/180	99.5	146/150	97.3	>	>
Do.	7°	7-12	17/20	85.0	17/20	85.0	<	<
Do.	12°	2-6	280/280	100.0	185/220	84.0		<
Do.	12°	7-12	20/20	100.0	20/20	100.0		
Do.	17°	7-12	18/20	90.0	16/20	80.0	<	<

[1] > = deeper anesthesia; < = similar to controls.

important effects of pH 5.0 to 8.5 on the sedating properties of MS-222.

Sedation is not a static stage of anesthesia. The stage shifts toward recovery, or deeper anesthesia, according to the concentration of anesthetic, temperature, and, in some instances, the size of the fish. The individuals in nearly 70 percent of the trials conducted at 17° C. showed signs of recovery after 6 hours. The trend was never toward deeper anesthesia in tests at this temperature. The shift at 7° and 12° was toward recovery in 40 percent of the tests, and toward deeper anesthesia in 20 percent. Sedation persisted in the remaining 40 percent. The reduced effectiveness of the drug at relatively higher temperatures is probably related to its deterioration, increased metabolism by the fish, or both. More conclusive evidence regarding the temperature dependence of MS-222 was obtained by McFarland (1959, 1960). His observations on the behavior and metabolism of anesthetized California killifish demonstrate that the drug was an effective sedative at 18° to 21° C., but not at 24° to 27° C. Sedation at the lower temperature range was not maintained beyond 6 to 8 hours.

One of the principal uses of anesthetics in the transportation of fish is to control metabolism. Baudin (1932a, 1932b), McFarland (1959), and Blahm (1961) reported that MS-222 reduces the rate of oxygen consumption in fish. We observed that the drug reduced the oxygen consumption of rainbow trout in a closed system by approximately 30 percent (table 8). Statistical analyses indicated that the reduction was significant at the 0.05 but not at the 0.01 level. In contrast, the anesthetic failed to reduce significantly the oxygen consumption rate of lake trout in open-system tests (table 9). This may have been the result of the differential diffusion of oxygen into those solutions with greater oxygen gradients. Thus, there is little advantage in using MS-222 in open systems to reduce oxygen consumption. On the other hand, the fish in these experiments were not purposely stimulated to higher levels of activity. Fry (1957) demonstrated that the oxygen uptake of stimulated fish may be four to five times the basal metabolic rate. The drug may be efficacious in open systems by lowering metabolic rates of this magnitude. McFarland (1960) suggested the pre-sedation of fish for several hours to lower their metabolic rates before transport.

TABLE 8.--Oxygen consumption of 6-inch rainbow trout sedated with MS-222 in closed systems at 12° C.

Test	Rates of oxygen consumption (mg.O_2/kg. body wt./hr.)	
	Sedated fish	Control fish
[illegible]	164.4	[illegible]
[illegible]	103.7	[illegible]
[illegible]	142.6	[illegible]
[illegible]	138.7	[illegible]
[illegible]	83.2	[illegible]
[illegible]	272.0	[illegible]
[illegible]	137.9	[illegible]
[illegible]	178.0	[illegible]
[illegible]	62.2	[illegible]
[illegible]	142.5	210.
[illegible]	57.7	57.1
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

*Significant at the 0.05, but not at the 0.01 level.

The reports on the benefits of sedating fish with MS-222 during transport are contradictory (Webb, 1958; Martin and Scott, 1959; Thompson, 1959; Meehan and Revet, 1962; Dollar, 1963; Rodman, 1963; Gebhards, 1965). The drug has been used more successfully at low, than at high temperatures, and it may be instrumental in reducing injuries to fish because of hyperactivity (Gossington, 1957; McFarland, 1960; Collins and Hulsey, 1963).

Use of MS-222 as a spawning aid

Anesthetization of gravid rainbow trout and brook trout with MS-222 was observed at three hatcheries. The rainbow trout were treated at national fish hatcheries at Genoa, Wis., and Manchester, Iowa, and the brook trout were exposed at the state fish hatchery at Osceola, Wis. The concentrations of drug were those normally used at the hatcheries during the collection of eggs and sperm. The observations are listed in table 10.

The amounts of MS-222 used to anesthetize the sexually mature, adult rainbow trout were similar to those producing rapid and moderately rapid anesthesia in the efficacy tests (table 4 and 6). A concentration of 58 p.p.m. produced loss of reflex in about 6 minutes, and it was as effective on fish weighing 8 pounds as on smaller individuals. The fish remained in this concentration for 6 to 28 minutes, and all recovered in 3 to 5 minutes. The desirable feature of this dosage was that a greater number of fish could be anesthetized over a longer period than would have been possible using higher dosages. Twenty fish were exposed in one trial and some remained in the anesthetic for nearly 30 minutes. They were not completely anesthetized and reacted slightly when handled. The more susceptible individuals were spawned within 5 minutes after contact with the anesthetic.

The fish exposed to 100 p.p.m. reached loss of reflex in 1.25 and 3.25 minutes. The duration of exposure was 2 to 7 minutes, and all recovered in 1 to 3 minutes.

The 166 p.p.m. of MS-222 used for male brook trout were higher than those employed in our efficacy tests (table 4). They were exposed for 4.25 to 7.5 minutes and all recovered in 7 to 9 minutes. The hatchery personnel reported that as many as 150 fish are anesthetized in one solution of MS-222 before

TABLE 9.--The oxygen consumption of 4-inch lake trout sedated with MS-222 in open systems at 12° C.

Elapsed time	Sedated fish			Control fish		
	Concentration of oxygen (p.p.m.)	Consumption of oxygen		Concentration of oxygen (p.p.m.)	Consumption of oxygen	
		p.p.m./hr.	mg.O_2/kg. body wt./hr.		p.p.m./hr.	mg.O_2/kg. body wt./hr.
0 hours	9.8	--	--	9.4	--	--
1.25 hours	8.3	1.2	80.0	6.8	2.1	140.0
1.5 hours	7.0	1.9	126.7	6.4	2.0	133.3
2 hours	6.5	1.7	113.3	6.2	1.6	106.7
2.5 hours	5.6	1.7	113.3	4.7	1.9	126.7
3 hours	5.2	1.5	100.0	4.6	1.6	106.7
3.5 hours	5.0	1.4	93.3	4.4	1.4	93.
4 hours	4.2	1.4	93.3	4.0	1.4	93.3
4.5 hours	3.6	1.4	93.3	3.0	1.4	93.3
Mean	.....	1.5	101.7	.....	1.7	111.6
Standard deviation	.....	.....	15.0	.....	.....	19.1
Difference between mean rates	9.9					
Calculated "t"	1.1					

TABLE 10.--Observations on use of MS-222 in anesthetizing rainbow trout and brook trout during spawning operations

Species and hatchery	Concentration (p.p.m.)	Temperature (F.)	Mean weight per fish (pounds)	Number of fish per test	Number of tests	Sex	Time (minutes) to reach loss of--		Range of exposures (minutes)	Recovery in fresh water	
							Equilibrium, stage 2	Reflex		Percent	Time (minutes)
Rainbow trout:											
Manchester, Ia.	58	54°	7.0	10	1	F	4.00	N.R.[1]	6.00- 8.00	100	3.00
Do.	58	54°	7.0	6	1	F	5.00	N.R.[1]	6.00- 8.00	100	4.00
Do.	58	54°	7.0	11	1	F	5.50	N.R.[1]	6.00- 8.00	100	3.00
Do.	58	54°	1.0	20	1	M	3.25	6.00	7.00-28.00	100	3.00-5.00
Do.	58	54°	1.0	15	1	M	5.50	N.R.[1]	N.R.[1]	100	N.R.[1]
Seneca, Wis.	100	51.5°	0.9	2	3	F	1.00-1.50	1.50-3.25	2.00- 5.00	100	2.50-4.50
Do.	100	51.5°	1.0	2	6	M	1.00-2.00	1.25-3.00	3.50- 7.00	100	1.00-3.00
Brook trout:											
Oneida, Wis.	110	48°	1.6	2	2	F	0.75	1.25-1.50	2.00- 2.50	100	2.00
Do.	110	48°	1.6	7	2	F	1.00-1.50	2.25-3.25	3.75- 5.75	100	8.00-9.75
Do.	166	48°	1.4	2	2	M	1.00	1.25-2.00	4.25- 5.00	100	7.00-8.00
Do.	166	48°	1.4	7	2	M	1.00	2.00-2.25	4.00- 7.50	100	8.00-9.00

[1] Not recorded.

its effectiveness is greatly reduced. Meister and Ritzi (1958) reported that from 22 to 35 pounds of brook trout, 36 to 86 pounds of Atlantic salmon, or 93 pounds of lake trout could be anesthetized per gram of MS-222.

The use of MS-222 during spawning speeds the operation and reduces the mortality of fish due to forceful handling. The anesthetic apparently has no adverse effects on the survival of eggs or parents (Parkhurst and Smith, 1957; Crawford and Hulsey, 1963). The exposed fish, however, are usually dipped into clean water before stripping to prevent the anesthetic from contacting reproductive products (Allison, 1961).

Other observations on MS-222

Minor deviations in the efficacy of MS-222 were observed from time to time. We believe that they were partially related to variations in the susceptibilities of individuals and groups of fish, or combinations of unknown factors. In any case, it is advisable to conduct preliminary bioassays of anesthetic solutions with several individuals from the stocks of fish which are to be narcotized. The precaution was recommended also by other investigators including Klontz (1964), Lamarque (1964) and Bell (1964). Observations on the depths of anesthesia and exposure times can be used to determine the numbers of fish which can be anesthetized safely.

CONCLUSIONS

Anesthetization of rainbow trout, brown trout, brook trout, and lake trout with MS-222 produced behavioral responses which were similar to those reported for other anesthetics and species of fish.

Concentrations of MS-222 which produced rapid anesthesia in the four salmonids ranged from 80 to 135 p.p.m. Levels of 50 to 60 p.p.m. induced moderately rapid anesthesia, and 15 to 30 p.p.m. were effective for sedation.

The action of MS-222 was reversible when the fish were removed from anesthetic solutions prior to the cessation of respiratory activity (medullary collapse). The retention of fish in solutions which are effective for rapid and moderately rapid anesthesia may result in mortality depending on the duration of exposure.

Brook trout and lake trout were more resistant to MS-222 at 7° and 12° C. than the other salmonids. Lake trout could withstand only short exposures and they died quickly after entering medullary collapse.

Effective concentrations and durations of exposure, particularly for rapid anesthesia, were inversely related to temperature. The rapid anesthetization of fish is more safely induced at lower temperatures.

There was little relation between size of fish and the efficacy of MS-222; however, the smaller fish occasionally had shorter exposure times.

The anesthetic solutions which contained 10 p.p.m. total hardness were less effective in narcotizing rainbow trout than those with hardnesses of 35 and 180 p.p.m. The fish anesthetized in soft water recovered sooner.

The trout which were repeatedly anesthetized with MS-222 were slightly more tolerant than previously unexposed individuals. The tolerance, however, did not increase with repeated treatments.

Sedation was not a static stage of anesthesia, but shifted toward recovery, or deeper anesthesia depending on the concentration of anesthetic and temperature.

MS-222 did not reduce the rate of oxygen consumption of lake trout in open systems. The consumption rate of rainbow trout in closed systems was reduced by approximately 30 percent. The efficacy of MS-222 was not altered to a measurable degree by pH 5.0 to 8.5.

Our results and those of other investigators demonstrate that MS-222 is effective and safe for inducing various stages of anesthesia in the four salmonids.

SUMMARY

Investigations were made to determine the concentrations of MS-222 which produce rapid, moderately rapid, and sedating anesthesia in rainbow trout, brown trout, brook trout, and lake trout. Eighty to 135 p.p.m. of MS-222 rapidly anesthetized the fish within 3 minutes at temperatures of 7° to 17° C. The fish could be exposed to the anesthetic for approximately 4 to 12 minutes. The effective concentrations and exposure times varied with species and temperature. Fifty to 60 p.p.m. induced a moderate rate of anesthesia which could be maintained for approximately 30 minutes. Sedation was produced within 15 minutes and maintained for 5 to 6 hours at 15 to 30 p.p.m. The efficacy of sedating concentrations appeared to decrease with time at 17° C. Lake trout required higher dosages of MS-222 than other salmonids for complete anesthesia, but they tolerated only short exposures.

There was no relation between size of fish and efficacy of MS-222. Smaller fish, however, occasionally had shorter exposure times. The efficacy of the drug was not influenced by pH 5.0 to 8.5.

Anesthetic solutions with a total hardness of 10 p.p.m. were less effective in anesthetizing rainbow trout than those containing 35 to 180 p.p.m. Individuals which were anesthetized in soft water recovered sooner.

Repeatedly anesthetized trout were slightly more tolerant than previously unexposed fish. Tolerance did not increase with repeated treatments.

Sedating concentrations of MS-222 did not lower the rate of oxygen consumption of lake trout in open systems. In closed systems, the consumption rate of rainbow trout was reduced approximately 30 percent.

We concluded that MS-222 is an effective and safe anesthetic for inducing anesthesia in the four salmonids. The relative safety of the drug was greater at lower temperatures. Our results and those of other investigators indicate that preliminary bioassays of anesthetic solutions are advisable to determine the desired rates of anesthesia and exposure times for specific conditions and lots of fish. Close observance on stages of anesthesia is essential to prevent excessive exposure of the fish at high concentrations.

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14. Method for Determining MS-222 Residues in Fish

By Charles R. Walker and Richard A. Schoettger

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METHOD FOR DETERMINING MS-222 RESIDUES IN FISH

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Abstract.--MS-222 is a primary aromatic amine. Its diazonium salt reacts with the Bratton-Marshall reagent (N-1-naphthylethylenediamine dihydrochloride) to form a wine-red azo dye with a maximum absorbance at 545 millimicrons. The diazotization reagent had to be modified and the reaction time extended to obtain a measurable yield of the diazonium salt. The regression of absorbance values versus concentrations of MS-222 which ranged from 0 to 7.5 p.p.m. had a slope of 1 and adhered to Beer's law. MS-222 was spiked into samples of blood, muscle, liver, and kidney from rainbow trout, and excellent recoveries were measured by the analytical method. The residues had to be distinguished from a background of primary aromatic amines which varied in each tissue, between individual specimens and with each lot of fish. Backgrounds were higher in liver and kidney, and residues of MS-222 were more difficult to differentiate. The method was particularly effective in determining residues of the drug in muscle and blood of trout.

MS-222 (tricaine methanesulfonate) is widely used as a fish anesthetic. Residues which might occur in fish flesh following use of the drug must be ascertained for safety of human consumers. Development of adequate information on residues required a method capable of measuring finite concentrations in the tissues.

A primary consideration in the acceptance of any analytical method for residues is the capacity to detect them at levels related to their toxicity to mammals. A contract study done for us by the Wisconsin alumni Research Foundation in March 1965 indicated that the oral LD_{50} of MS-222 for Sprague-Dawley strain laboratory rats is between 5 and 10 grams per kilogram of body. Thus, the drug is relatively nontoxic to mammals according to the standards of the American Industrial Hygiene Association (Spector, 1956). An analytical procedure capable of defining residues in parts per million should be adequate for all practical purposes.

Friddle and Snieszko (1950), using the Bratton-Marshall (1939) method, found that MS-222 caused an error in their evaluation of sulfa residues in anesthetized brook trout. We believe this can be explained by the fact that MS-222 is a primary aromatic amine and reacts readily in the colorimetric procedure. We chose to investigate the applicability of this colorimetric reaction to determinations of MS-222 in fish tissues.

METHODS AND MATERIALS

Experiments on operating parameters

Diazotization of the m-aminoethylbenzoate constituent of the MS-222 salt was accomplished by modification of the procedure described by Bratton and Marshall (1939). The conditions for formation of the diazonium ion and the subsequent reaction with the coupling reagent were established in a series of experiments.

The diazonium salt was obtained in a 5-ml. sample containing MS-222, m-aminoethylbenzoate, by adding 0.5 ml. of 0.2-percent sodium nitrite. The excess nitrous acid was destroyed by ammonium sulfamate. The diazonium salt was reacted with the Bratton-Marshall reagent, N-1-naphthylethylenediamine dihydrochloride, to form the azo dyestuff.

The effect of diazotization time upon the concentration of azo dye formed was measured in 40 tests involving 160 reactions made with 0, 1, 3, 5, or 7.5 p.p.m. of MS-222 standards. Diazotization reactions were stopped at 3, 5, 7 to 10, and 15 to 30 minutes. The percent transmittance values were plotted for each concentration and the regression was calculated for each time interval.

The speed of azo dye formation upon addition of the coupling reagent was measured at 0, 1, 3, and 5 p.p.m. of MS-222 in standards reacted at room temperature following diazotization. The transmittance values were recorded at 30-second intervals for the first 5 minutes, 1-minute intervals for the next 10 minutes, and at 5-minute intervals up to 1 hour to measure the effect of development time.

The effect of temperature on the diazotization reaction and the resultant concentration of azo dye formed was tested at 15°, 22.5°, and 30° C. Twenty tests were conducted at the longer diazotization times at each 1-minute interval from 10 minutes through 30 minutes for each concentration of 1, 3, and 5 p.p.m. of MS-222. The mean transmittance value for each concentration and temperature was calculated for 5 measurements taken at 10-15 minutes, for 5 at 15-20 minutes, and for 10 between 20 and 30 minutes.

The maximum limit for the reaction of the diazonium ion was between 5 and 7.5 p.p.m. of MS-222 without causing precipitation on the sides of cuvettes and or optical density less than 20 percent. Thus, dilutions were designed to keep the concentration in samples in the working range of the instrumentation.

Measurements of the maximum absorbance value of the azo dye at 420 to 650 millimicrons were made with a Beckman DB spectrophotometer and a Sargent SRL recorder. Mechanical Adjustments for slit width for the maximum absorbance band were established on standard solutions of MS-222 at concentrations up to 7.5 p.p.m. with a Perkin-Elmer 139 spectrophotometer equipped with a photomultiplier.

Fish

We used rainbow trout in the tests of the residue method. The 8- to 14-inch fish were furnished by the Manchester, Iowa, National Fish Hatchery and were held in facilities of the Fish Control Laboratory. They were fed a pelleted, open-formula diet. It is important to point out that several ingredients of the diet were fortified with primary aromatic amines or vitamins such as folic acid, vitamin B_{12}, p-aminobenzoic acid plus protein sources such as brewer's yeast.

Tissue collection and treatment

Blood samples were drawn from specimens at specified intervals by cardiac puncture with a heparinized syringe and 18- or 20-gage needle. Within 15 minutes after the blood was taken, the samples were mixed with trichloroacetic acid (TCA) for coagulation of protein.

Each fish was immobilized by a blow on the head or pithing and the other tissues were dissected out. Skin was removed to expose the thick muscular area adjacent to the dorsal fin. A sample about 1 inch square was removed, minus bones, and placed in a tared beaker and weighed. It was homogenized and diluted 1:20 with TCA and distilled water to make up a 3-percent TCA solution.

The entire liver, excluding the gall bladder, was removed. Kidney tissue was dissected posterior to the isthmus. The tissues were weighed in tared beakers and those not processed immediately were quick-frozen with dry ice. The tissues were processed for chemical analysis in the same manner as muscle.

Analysis of residues

The concentration of aromatic amines and other masking or interfering substances were determined for blood, muscle, kidney and liver in 9 specimens of rainbow trout and expressed in terms of parts per million of MS-222. The recovery of MS-222 from each tissue was evaluated by spiking at rates of 0, 20 and 100 p.p.m. in each lot of 3 fish by using the outlined procedure.

The colorimetric reaction described by Bratton and Marshall (1939) was used, with modifications, for detection of MS-222 in fish tissues. The process was as follows:

Reagents:

1. 15-percent trichloroacetic acid: Dissolve 30 g. crystalline TCA in distilled water. Transfer to a 200-ml. volumetric flask and bring to volume with distilled water. Dilute 20 ml. to 100 to get 3-percent TCA.

2. 0.2-percent sodium nitrite: Dissolve 0.5 g. $NaNO_2$ in water. Transfer to a 100-ml. volumetric flask and bring to volume. Transfer 40 ml. to a second 100-ml. volumetric flask and bring to volume. Make fresh daily.

3. 0.5-percent ammonium sulfamate: Dissolve 0.5 g. in distilled water and dilute to 100 ml.

4. 0.1-percent N-1-naphthylethylenediamine dihydrochloride: Dissolve 0.5 g. in distilled water and bring to 100 ml. Transfer 20 ml. to 100-ml. volumetric flask and bring to volume. Refrigerate and make up fresh weekly.

5. 4N hydrochloric acid: Dilute 40 ml. concentrated HCl to 100 ml. Mix and titrate a 5 ml. portion with standard 1N NaOH. Adjust to exactly 4N.

6. 10-percent HCl.

Standards:

1. Stock solution: Weigh 0.1000 g. of MS-222 and dissolve in distilled water. Quantitatively transfer to 100-ml. volumetric flask, add 20 ml. of 15-percent TCA and dilute to volume with distilled water. Stock equals 1,000 p.p.m. of MS-222 in 3-percent TCA.

2. Dilute 2.0 ml. stock to 200 ml. with 3-percent TCA. This stock contains 10 p.p.m. of MS-222.

3. Volume of 10 p.p.m. stock is diluted to 50 ml. with 3-percent TCA:

Desired p.p.m.	ml. of 10-p.p.m. stock
9.0	45.0
7.5	37.5
5.0	25.0
3.0	15.0
1.0	5.0
0.5	2.5
0.25	1.25

Procedure:

1. Sample preparation--blood and tissue.

   A. Blood: Use an anticoagulant, and draw blood with care to avoid contamination with body fluids or water. Place 0.5 ml. in 7.5 ml. of distilled water and let stand 10 minutes or until other samples are ready. Add 2 ml. of 15-percent TCA. Mix thoroughly and let set 5 minutes. Centrifuge at 2,500 RPM for 30 minutes. Filter to remove fat or other materials not spun down. Go to Part 2, Analysis.

   B. Other tissue (muscle, kidney and liver): Carefully dissect out the sample, weigh, homogenize and dilute in the following manner: One gram of tissue is homogenized and diluted to 16 ml. with distilled water in a graduated centrifuge tube. A 1:20 dilution of the sample is achieved by adding 4 ml. of 15-percent TCA. The precipitate of protein is removed by the centrifugation and filtration outlined for blood as above. The supernatant is then analyzed according to part 2, Analysis.

2. Analysis.

   A. Pipette 5 ml. of filtrate into a clean 25-ml. Erlenmeyer flask.

   B. Pipette 5 ml. of 3-percent TCA into another flask as a reagent blank.

   C. Add 0.5 ml. of 0.2-percent $NaNO_2$ to each flask, swirl, and let stand 15 minutes. Keep out of direct sunlight.

   D. Add 0.5 ml. of 0.5-percent ammonium sulfamate to each flask, swirl and let stand 2 to 3 minutes.

   E. Add 0.5 ml. of 0.1-percent N-1-naphthylethylenediamine dihydrochloride to each tube or flask. Swirl for 30 to 60 seconds and let stand 5 or 10 minutes. Keep out of direct sunlight.

   F. Pipette or pour into cuvettes and read in spectrophotometer at 545 millimicrons. A Model 139, Perkin and Elmer spectrophotometer with a slit width of 0.05 mm. and sensitivity of X1 was used. Sensitivity of the photomultiplier was set at 5. Obtain p.p.m. or microgram from precalibration curve or use a standard. Correct for dilution and quantity of tissue. The concentration will be in terms of free MS-222.

   G. Possible acetylated derivatives: Place 10 ml. of filtrate into a graduated centrifuge tube. Add 0.5 ml. of 4N HCl. Place in a boiling water bath for 1 hour, cool, and adjust volume to 10 ml. with water. Transfer 5 ml. to a 25-ml. Erlenmeyer flask and treat as above for free MS-222.

3. Other considerations.

   A. Bubble formation: Occasionally bubbles form in cuvettes. The cuvettes should be capped and slowly tipped. Most of the bubbles on the transparent surface should be absorbed by the large air bubble in the cuvette. The use of larger Erlenmeyer flasks may also reduce bubble formation.

   B. Glassware cleanliness: Flasks and cuvettes which have been used in steps E and F should be rinsed, first in 10-percent HCl and then in distilled water. A colored residue may adhere to the glassware and produce errors in subsequent analyses. Several rinsings with acid are usually sufficient to remove the residue.

RESULTS

Chemistry of the colorimetric reaction

The majority of applications of the Bratton-Marshall reaction employing the N-1-naphthylethylenediamine coupling reagent have involved para-substituted compounds such as sulfonamides, procaine hydrochloride, p-aminobenzoic acid, folic acid, and chloramphenicol (Meites, 1963; Welcher, 1963; Varley, 1963). However, coupling of diazonium salts of both para- and ortho-primary aromatic amines are widely illustrated in the chemistry of azo dyestuffs (Brewster and McEwer, 1961). In our application, we are diazotizing a primary aromatic amine in the meta position to a carboxyl group. The preparation of the diazonium salt of MS-222 would probably follow the flow scheme illustrated by Natelson (1961) (fig. 1).

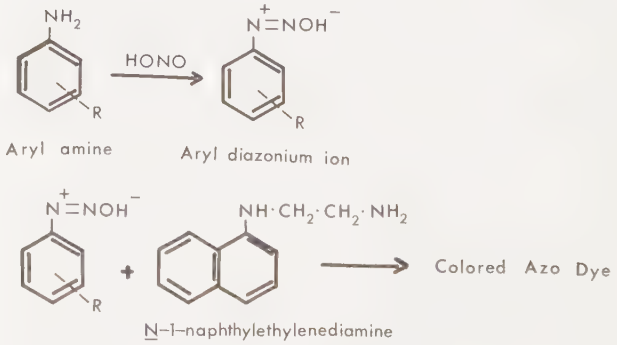

Figure 1.--Probable flow scheme of the diazotization of the ethyl ester of m-aminobenzoate (MS-222) and the coupling reaction with N-1-naphthylethylenediamine after Natelson (1961). The HCl maintains the pH at 1 to 2.

Apparently, this reaction proceeds much slower with the meta isomer than with ortho and para isomers. The suggested time for diazotization of para-substituted sulfonamides was 3 minutes with 0.1-percent sodium nitrite reagent (Bratton and Marshall, 1939). The diazotization of MS-222, however, required at least 0.2-percent sodium nitrite. A more consistent production of diazonium salt occurred at 15° to 30° C. in 15 to 30 minutes (table 1). Holbourn and Pattle (1943) observed the necessity for longer diazotization time in their work with sulfanilamide and the Bratton-Marshall reagent. They also noted the effect of light on the reaction and coupling with the reagent.

According to Marshall and Litchfield (1938) and Bratton and Marshall (1939), the ammonium sulfamate effectively destroys the excess nitrous acid. Compared to the reaction time indicated for the sulfa drugs, the diazonium salt of MS-222 appears to undergo nitroso formation at a relatively slow rate, thus providing more latitude of time prior to the termination of diazotization. Upon addition of the Bratton-Marshall reagent, N-1-naphthylethylenediamine dihydrochloride solution, the azo dyestuff formed rapidly and attained a maximum intensity within a few minutes (fig. 2). The azo dye coupling reaction proceeds immediately to form a wine-red colored azo dyestuff with a maximum absorbance in the spectral range of 545 millimicrons (fig. 3). This absorbance curve is very similar to that illustrated for sulfa drugs by Natelson (1961). The absorbance values or percent transmittance were plotted

TABLE 1.--Effect of diazotization times at three temperatures on the percentage of transmittance values observed for MS-222 in the Bratton-Marshall reaction

Diazotization time and temperature	Percentage of transmittance at--					
	1 p.p.m.		3 p.p.m.		5 p.p.m.	
	Mean	Range	Mean	Range	Mean	Range
10-15 minutes:						
15.0° C.	78.3	77.8-81.5	48.4	46.9-50.3	29.7	29.5-30.6
22.5° C.	77.8	76.5-80.4	47.7	45.0-48.4	28.7	27.5-30.2
30.0° C.	77.0	76.5-78.8	46.9	45.0-47.6	28.2	27.4-29.3
15-20 minutes:						
15.0° C.	77.2	76.8-79.1	47.0	46.3-50.0	28.1	27.6-29.5
22.5° C.	76.8	76.8-77.6	46.6	45.5-47.0	27.5	26.5-28.9
30.0° C.	76.3	75.7-77.0	46.0	45.0-46.7	27.0	26.3-27.9
20-30 minutes:						
15.0° C.	76.1	75.8-76.5	44.6	43.9-45.0	26.2	25.8-26.6
22.5° C.	76.6	75.0-76.1	44.0	43.3-44.4	25.8	25.2-26.2
30.0° C.	75.3	74.9-75.8	43.5	43.0-44.0	25.3	24.7-25.8

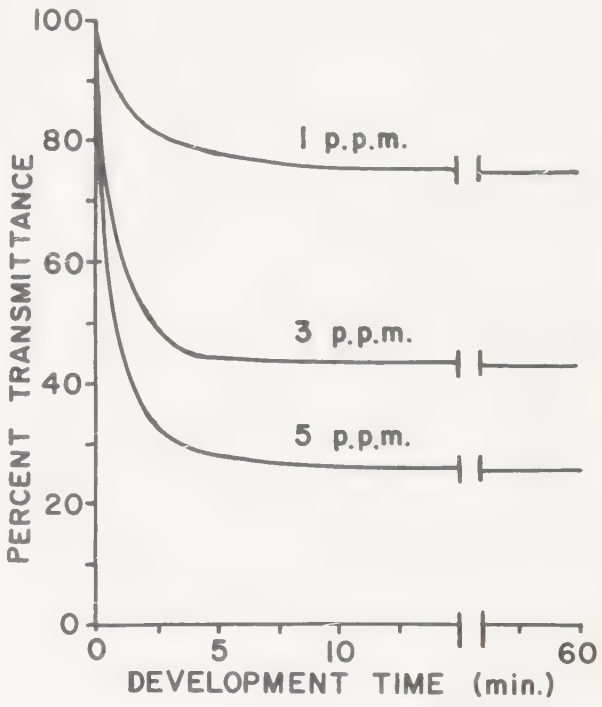

Figure 2.--Speed of development of the azo dyestuff in the coupling reaction of N-1-naphthylethylenediamine with three concentrations of diazotized MS-222.

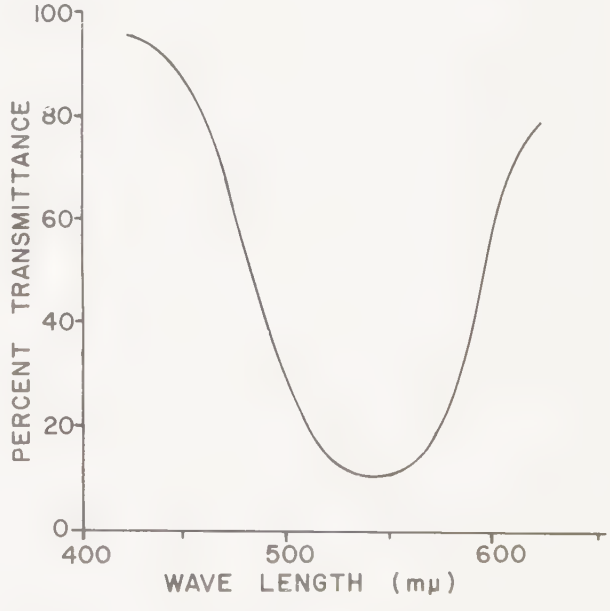

Figure 3.--Absorption spectrum for the azo dyestuff developed in the reaction of diazotized MS-222 and N-1-naphthylethylenediamine.

on the logarithmic scale versus the concentration in p.p.m. as MS-222 on an arithmetic scale with 1-cycle logarithmic paper (fig. 4). A straight line relation exists between means of points established at 1, 3, and 5 p.p.m., and it adheres to Beer's law over a wide range of diazotization times. Greater sensitivity was obtained at 15 to 30 minutes, and the values did not vary as greatly as those at shorter diazotization periods.

The accuracy of the determination is greatest in the middle section of the curve. Hawk et al. (1954) mention that higher concentrations produce colors too deep for precise measurement. The dyestuff appears to precipitate at the liquid-air interface at the higher concentration of MS-222, and it adheres to the sides of glassware. It is exceedingly important that cuvettes be rinsed with 10-percent HCl prior to introducing the developed sample. We also found it expedient to rinse the cuvettes at least once with the developed sample solution to avoid a negative dilution error. Contaminants and/or gas bubbles on the sides of cuvettes in the light path may produce serious quantitative errors at high transmittance. Particular care must be taken to remove detergent film from glassware by a thorough rinsing with dilute HCl and deionized water. Detergents cause yellow or green-brown turbidity in extreme cases of contamination. This turbidity is distinguishable from the nitroso formation of azoxy compounds of MS-222 and natural aromatic amines which are usually reddish-brown in color.

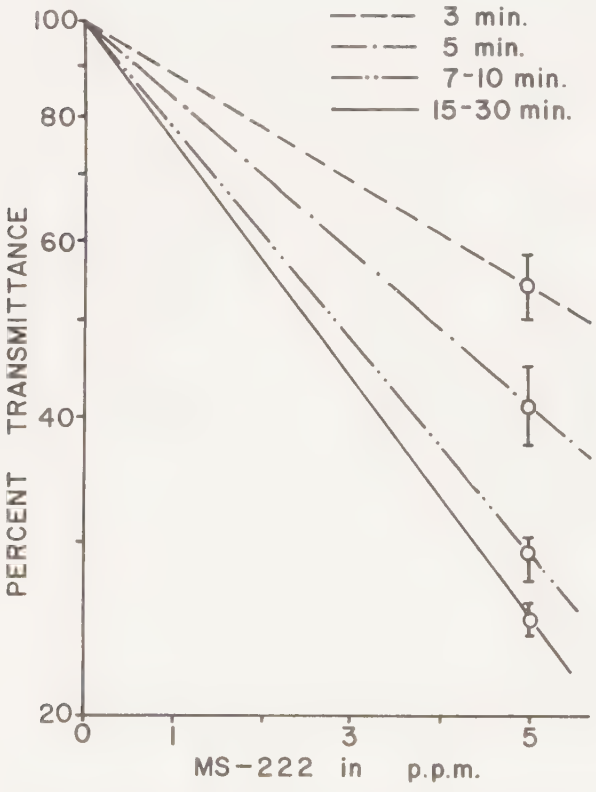

Figure 4.--Transmittance curves of MS-222 showing the effects of different diazotization times on the intensity of color development at 545 millimicrons and the range of values observed at 5 p.p.m.

Measuring MS-222 in fish

Since the Bratton-Marshall color reaction is specific to aromatic amines, the quantitation of MS-222 has to be differentiated from natural occurring amines such as p-aminobenzoic acid (Hawk et al., 1954). Thus, we determined background levels of amines and other masking substances in blood, muscle, kidney, and liver (table 2). Further, the amounts of recoverable MS-222 were determined by spiking samples of two concentrations (table 3). This determined its effectiveness in measuring different concentrations in the various tissues over and above background interferences. Recoveries ranged from 89 to 112 percent for all samples.

TABLE 2.--Concentrations of background at 545 millimicrons in rainbow trout

Tissue	Number of fish	Background		Standard error	95-percent confidence interval
		Mean (p.p.m.)	Range (p.p.m.)		
Blood.....	9	0.91	0.0- 2.0	0.19	0.46- 1.36
Muscle.....	9	0.87	0.0- 2.0	0.29	0.21- 1.53
Kidney.....	9	5.42	2.8- 9.0	0.58	4.08- 6.76
Liver.....	9	18.13	11.6-20.0	0.96	15.92-20.34

TABLE 3.--Recovery of MS-222 spiked into various tissues of rainbow trout

Tissue	Number of fish	Concentration of spikes (p.p.m.)	MS-222 in tissue		
			Mean (p.p.m.)	Range (p.p.m.)	Percent recovery
Blood.....	3	20	22.47	21.6- 23.8	112.3
	3	100	99.47	96.6-101.0	99.5
Muscle.....	3	20	21.00	20.6- 21.4	105.0
	3	100	101.87	94.2-106.2	101.9
Kidney.....	3	20	18.27	17.8- 18.6	91.3
	3	100	88.93	86.2- 91.6	88.9
Liver.....	3	20	20.40	19.2- 21.4	101.7
	3	100	103.30	95.2-109.6	103.3

Mooney and Pasarela (1964) refined the cleanup of samples to reduce contamination by procaine penicillin and other interfering substances in the background. They employed a Dow-50W-X2 (H^+) ion exchange resin to extract sulfonamides selectively in acid phase and to elute them in alkaline phase. This method was not applicable to the cleanup of fish tissues because we could not elute MS-222 from the column.

Liver gave the most interference by producing a true red, azo dye color reaction, and the levels were quite consistent with a small deviation and narrow confidence limits. Kidney samples were characterized by a yellow color. This pigment introduced a negative interference with the azo dye reaction, but gave a consistent recovery of spiked MS-222. Blood and muscle gave relatively little color reaction or interference. The volumes of blood samples, however, were small, and contributed to positive dilution errors. The errors were particularly significant in the recovery of lower concentrations of MS-222. In general, the overall accuracy of the method is governed by the techniques of extraction and dilution.

CONCLUSIONS

MS-222 can be detected in fish tissues by means of a modified Bratton-Marshall method. Interferring substances were more prevalent in liver and kidney than in blood and muscle. The recovery of MS-222 from spiked samples ranged from 89 to 112 percent. The method was more accurate for measuring MS-222 in blood and muscle than in kidney and liver.

SUMMARY

Use of MS-222 as a fish anesthetic necessitated an investigation into the residues in fish following exposure. Fortunately, the toxicity of MS-222 to mammals was found to be quite low with an acute oral LD_{50} of approximately 5-10 g./kg. to laboratory rats. Thus, an analytical procedure which would define concentrations in p.p.m. was considered adequate.

MS-222 has the chemical structure of a primary aromatic amine and it reacts colorimetrically with the Bratton-Marshall reagent. We modified the method commonly used for determination of sulfa drugs.

MS-222 forms a diazonium salt much more slowly than the para-substituted aromatic amines and thus required a higher concentration of nitrous acid and longer time for development. The azo dye coupling reaction proceeds almost immediately with N-1-naphthylethylenediamine dihydrochloride to form a wine-red dye complex with a maximum absorbance in the spectral range of 545 millimicrons. The regression of absorbance values was almost linear with a slope of 1.0, and it bore evidence of adherence to Beer's law.

The fish tissues were extracted in distilled water, and the protein was precipitated in a 3-percent TCA solution. The solution was centrifuged and then filtered to remove the lighter fatty fraction. A 1-to-20 dilution was necessary to adjust the concentration within the limits of the transmission curve (0 to 7.5 p.p.m.).

We obtained excellent recoveries of MS-222 from all spiked samples of fish tissue. They ranged from 89 to 112 percent which excluded the aromatic amines of natural origin. The background of primary aromatic amines varied with each tissue and between individual specimens. The backgrounds were higher in liver and kidney, and residues of MS-222 were more difficult to differentiate. In liver, it was due, presumably, to the diazotized amines such as vitamin B_{12}, p-aminobenzoic acid, folic acid, and the many proteins in fortified diets for trout. A yellow pigment persisted in extracts from kidneys which introduced a negative interference with the azo dye reaction. The modified Bratton and Marshall method was, however, particularly effective in determining residues of MS-222 in muscle and blood of trout.

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15. Residues of MS-222 in Four Salmonids Following Anesthesia

By Charles R. Walker and Richard A. Schoettger

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RESIDUES OF MS-222 IN FOUR SALMONIDS FOLLOWING ANESTHESIA

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Abstract.--Residues of MS-222 (tricaine methanesulfonate) in the blood, muscle, liver, and kidney of rainbow trout and in the muscle of brown trout, brook trout, and lake trout were measured by a modified Bratton-Marshall colorimetric method. Temperatures were 7°, 12°, and 17° C. in waters with total hardnesses of 10 to 180 p.p.m. The residues were easily detected and measured in blood and muscle, but they were masked in liver and kidney by background substances. The anesthetic dissipated rapidly in the muscle of the four species within 1 to 6 hours, and the residues approached the background levels of controls within 9 to 24 hours after withdrawal from exposure. The differences in residue levels in the muscle at 0-hour withdrawal between species, at three temperatures, and in soft and hard water largely disappeared within 24 hours.

MS-222 (tricaine methanesulfonate) has gained wide acceptance as a fish anesthetic and transportation sedative. There has been no investigation, however, on its residues in fish following treatment. This information is especially important in situations where fish exposed to anesthetic or sedating solutions are eaten soon after by people.

An extensive review of the literature on the many uses of MS-222 was made by Schoettger (1966). Schoettger and Julin (1966) defined the criteria for desired stages of anesthesia, and the efficacy of MS-222 at various concentrations and times of exposure to rainbow trout, brown trout, brook trout, and lake trout. The analyses of residues of the anesthetic in these species were performed concurrently with the investigations of efficacy.

We selected the extremes of concentration and depth of anesthesia to ascertain the existence and persistence of residues in the four trouts. The major emphasis was placed on the determination of residues in muscle since it is more important than other tissues in human consumption. The rainbow trout, the species more widely propagated for sport and food in the United States, was used for evaluation of residues in other tissues: blood, liver and kidney. We also concentrated observations at 12° and to lesser extent at 7° and 17° C. This range of temperature is considered to be the most suitable for trout production (Davis, 1956).

METHODS AND MATERIALS

Fish

The rainbow trout, brown trout, brook trout, and lake trout ranged from 7 to 14 inches long, and were obtained from State fish hatcheries at Lanesboro, Minn. and St. Croix, Wis., and from national fish hatcheries at Jordan River, Mich., Lake Mills, Wis. and Manchester, Ia. They were held in well water and facilities described by Lennon and Walker (1964) and taken off feed for

at least 24 hours before treatment with MS-222.

Anesthetization

Schoettger and Julin (1966) established the efficacy of MS-222 as an anesthetic for several species of trout at various temperatures and water qualities. Concurrent with these studies, we sampled tissues from the fish which were anesthetized at 7, 12 and 17° C. The concentrations of anesthetic solutions ranged from 80 to 135 p.p.m., and the mean time to reach the desired stage of anesthesia ranged from 4 to 12 minutes depending on temperature and species. After specimens reached deep anesthesia, they were withdrawn from the drug solution and placed in fresh water for recovery. This action marked the beginning of withdrawal time.

Collection and analyses of tissues

The methods used for collecting and analyzing the blood, muscle, liver, and kidney of trout were described by Walker and Schoettger (1966).

RESULTS

Preliminary analyses

Initially we sought to detect and measure residues of MS-222 in blood, muscle, liver, and kidney of rainbow trout at intervals of 0, 5, 10, 20, and 30 minutes after withdrawal from 9 minutes of exposure to 100 p.p.m. of drug at 12° C. The 12- to 14-inch fish were in advanced loss of reflex and some had reached medullary collapse (table 1). Residues occurred in all tissues and the higher concentrations were found in blood, liver, and kidney at 0-minute withdrawal. The higher concentration of residue in muscle was detected at the 10-minute withdrawal. The results were variable. It was apparent that withdrawal time should be extended beyond 30 minutes, more samples should be used, and all specimens should be in the same stage of anesthesia.

TABLE 1.--Residues of MS-222 including background amines recovered from adult rainbow trout following withdrawal from anesthesia

Fish sample	Interval after withdrawal (minutes)	Residues in p.p.m.			
		Blood	Muscle	Liver	Kidney
No. 1.....	0	61.6	7.3	49.5	91.5
No. 2.....	0	--	6.7	53.7	52.3
No. 3.....	5	17.6	1.3	21.3	29.2
No. 4.....	5	20.2	9.8	14.6	27.8
No. 5.....	10	13.0	18.9	25.4	23.9
No. 6.....	10	9.1	1.1	8.3	9.4
No. 7.....	15	6.9	1.1	37.4	9.6
No. 8.....	15	9.5	7.3	11.5	10.6
No. 9.....	20	7.1	9.2	41.3	14.9
No. 10.....	20	6.3	4.2	23.6	5.9
No. 11.....	30	5.9	4.0	11.5	8.7
No. 12.....	30	5.1	0.7	11.5	2.3

Another group of 15 rainbow trout, 12 to 14 inches long, was anesthetized in 100 p.p.m. of MS-222 at 12° C. to a state of medullary collapse. Tissues were sampled for residues at 0, 1, and 2 hours following withdrawal (table 2). The concentrations of the drug were highest in the blood of fish at 0-hour withdrawal. They dissipated rapidly as shown in a regression (fig. 1).

A similar pattern was noted in the muscle, although the initial concentration of residue was somewhat lower (table 2 and fig. 2). The regressions indicate that the amounts of MS-222 in blood and muscle approach background quantities of aromatic amines after 2 hours of withdrawal from the anesthetic solution. The 95-percent confidence intervals overlap at the 2-hour withdrawal.

The livers of untreated fish had a wide range in concentration of background amines, and it was difficult to distinguish between them and actual residues of MS-222 (table 2). The apparent concentration of the drug in liver was highest at the 1-hour reading and fell off in 2 hours to values only slightly above controls. An extrapolation of the regression indicates that residues of the drug persist beyond 5 hours (fig. 1).

The residues of MS-222 in kidney were somewhat more persistent than those in muscle but also declined during the 2-hour period following withdrawal (table 2). A projection of the regression makes an interception very close to the 2-hour reading (fig. 1). The broad confidence intervals at the 1- and 2-hour withdrawals overlap

TABLE 2.--Residues of MS-222 including background amines recovered from adult rainbow trout following withdrawal from anesthesia

Tissue and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
Blood:							
Control	5	13.4	425.0	2.60	1.2- 4.2	0.56	1.
0 hour	5	13.2	412.4	69.40	63.4-78.4	3.11	60.
1 hour	5	12.6	319.0	15.88	4.3-28.2	0.56	14.
2 hours	5	12.8	348.0	5.96	4.0-10.0	1.09	2.
Muscle:							
Control	5	13.4	425.0	1.92	0.8- 3.6	0.50	0.53- 3.31
0 hour	5	13.2	412.4	7.52	2.2-11.4	1.61	3.06-11.98
1 hour	5	12.6	319.0	4.28	2.6- 7.6	0.63	2.53- 6.03
2 hours	5	12.8	348.0	3.52	2.6- 4.6	0.32	2.63- 4.41
Liver:							
Control	5	13.4	425.0	38.44	31.0-50.2	3.3	
0 hour	5	13.2	412.4	46.00	34.6-71.0	7.0	
1 hour	5	12.6	319.0	52.68	41.0-67.4	4.62	
2 hours	5	12.8	348.0	43.00	29.4-63.0	5.99	
Kidney:							
Control	5	13.4	425.0	15.32	11.8-16.2	0.88	12.88-17.7
0 hour	4	13.1	408.5	55.85	47.8-70.0	5.10	39.61-72.0
1 hour	4	12.6	315.8	24.20	16.0-46.4	7.41	0.64-47.76
2 hours	5	12.8	348.0	17.20	14.2-19.2	0.82	4.92-19.48

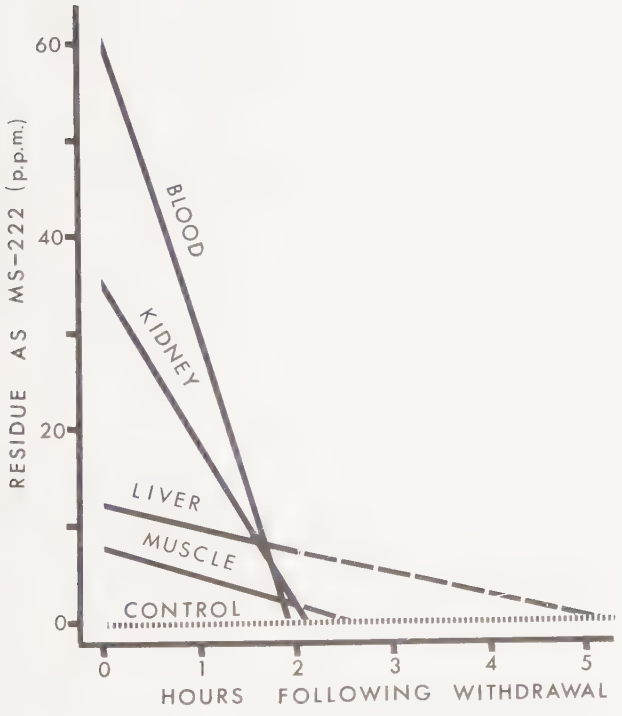

Figure 1.--Regressions of the residues of MS-222 in blood, muscle, liver, and kidney of rainbow trout on withdrawal time. The residues are expressed as concentration above the mean background value for control fish.

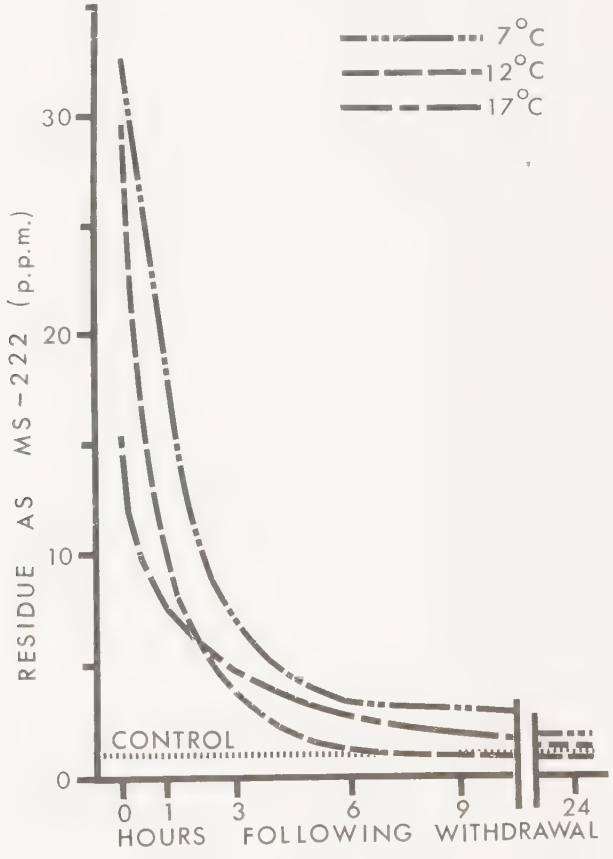

Figure 2.--Regression of the residues of MS-222 in muscle of four species of trout on withdrawal time following deep anesthesia at three temperatures. The lines were drawn through mean values for residues at each withdrawal.

considerably. We also found that the high levels of background in untreated controls differed slightly between these time intervals.

The uptake of MS-222 was higher in individuals which were anesthetized in soft water than those treated in hard water (table 3). The greater uptake of drug in soft water appeared to be related to longer exposures. The times

TABLE 3.--Residues of MS-222 including background in muscle of rainbow trout following deep anesthesia in hard and soft water at 12° C.

Hardness and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
Hard water (180 p.p.m.):							
0 hour	3	11.5	233.3	9.60	3.4-16.0	2.10	0.99-18.21
24 hours	3	10.6	188.3	2.37	2.1- 2.6	0.09	2.01- 2.73
Soft water (10 p.p.m.):							
0 hour	3	11.5	233.3	30.73	27.6-35.6	1.43	24.39-36.57
24 hours	3	11.1	216.3	2.47	2.2- 2.8	0.10	2.03- 2.90

for anesthetization of fish in soft water were approximately double those in hard water, but recoveries were more rapid (Schoettger and Julin, 1966). The concentrations of acetylated MS-222 in fish anesthetized in soft and hard water were approximately the same. However, they amounted to 20 percent of the total aromatic amines which were measured in fish treated in soft water as compared with 40 percent for fish in hard water at the 0-hour withdrawal. We were unable to show any differences in concentrations of free MS-222 at 24 hours, and approximately 30 to 35 percent of the total aromatic amines were acetylated.

TABLE 4.--Residues of MS-222 including background in blood of rainbow trout following deep anesthesia at selected time intervals and water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error
		Length (inches)	Weight (gram)	Mean	Range	
7° C.:						
0 hour	2	10.00	154.0	52.8	39.8-65.8	13.00
6 hours	2	9.70	131.5	2.5	2.0- 3.0	0.50
9 hours	2	9.95	141.0	1.6	1.4- 1.8	0.20
24 hours	1	10.00	154.0	1.0	--	--
12° C.:						
0 hour	2	9.85	141.5	60.6	57.2-64.0	3.40
1 hour	2	10.00	150.5	6.2	5.6- 6.8	0.60
3 hours	2	9.80	150.0	2.1	2.0- 2.2	0.10
6 hours	1	10.10	155.0	2.6	--	--
9 hours	2	9.25	118.0	1.9	1.8- 2.0	0.10
17° C.:						
0 hour	2	10.55	187.0	45.8	43.8-47.8	2.00
6 hours	2	10.15	159.5	5.1	4.8- 5.4	0.30
9 hours	2	10.25	166.5	4.6	4.6- 4.6	0.00
24 hours	2	10.40	168.0	1.4	1.0- 1.8	0.40

Intensive analyses on four species

Rainbow trout.--Seventy-five 8- to 12-inch rainbow trout were anesthetized to medullary collapse in 100 p.p.m. of MS-222 at 7°, 12°, and 17° C. (table 4). The induction of anesthesia ranged from 6.8 to 11.4 minutes at 7°, 8.0 to 11.9 minutes at 12°, and 5.5 to 6.9 minutes at 17° C.

The residues including background in blood at 0-hour withdrawal ranged from 39.8 to 65.8 p.p.m., with the least variation at the higher temperature (table 4). They decreased rapidly at 7° and 12° compared with 17° C. The levels of background in these tests were 0.8 to 1.0 p.p.m. (table 5). Thus, the residues of MS-222 approach the background levels after 24 hours withdrawal.

The highest concentrations of residues of MS-222 including background in muscle occurred at 0-hour withdrawal at all water temperatures (table 6). They varied greatly between individual fish, thereby causing large standard errors and making the calculation of 95-percent confidence intervals impractical. The standard errors decreased as the time of withdrawal increased. Furthermore, the residues of 9.4 to 72.0 p.p.m. at 0-hour withdrawal declined rapidly to a range of 0.8 to 3.8 p.p.m. at 6 hours.

The mean residues in muscle at the 9-hour withdrawal were equal to or less than background values, and the confidence intervals were similar (table 5). It appears that no significant residues of MS-222 remain in muscle at 9 hours after withdrawal.

The residues of MS-222 in livers exceeded the mean background by 17 to 59 p.p.m. at 0-hour withdrawal (table 7). Thereafter, the residues were masked by the high and variable levels of background amines (table 5).

The residues in kidneys at 0-hour withdrawal exceeded the mean background by 21 to 90 p.p.m. (table 8). At 7° and 17°, the mean residues at 9- to 24-hour withdrawals were below the means of background in untreated controls (tables 5 and 7). At 12°, the mean residues beyond 3 hours of withdrawal fell within the range of background values.

TABLE 5.--Background residues of aromatic amines in tissues of fish which were used as untreated controls in the study of MS-222.

Species and tissue	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
Rainbow trout:							
Blood	2	9.22	110.80	0.90	0.8- 1.0	0.22	--
Muscle	5	9.22	110.80	1.44	0.8- 2.2	0.26	[illegible]
Liver	5	9.22	110.80	13.72	11.6-16.0	0.72	[illegible]
Kidney	5	9.22	110.80	7.12	2.6-10.4	1.36	[illegible]
Brown trout: Muscle	3	7.60	71.67	0.40	0.2- 0.6	0.12	0.00- 0.92
Brook trout: Muscle	3	9.53	137.33	0.53	0.4- 0.6	0.07	0.23- 0.83
Lake trout: Muscle	3	7.60	45.67	0.40	0.2- 0.6	0.11	0.00- 0.87

TABLE 6.--Residues of MS-222 including background in muscle of rainbow trout following deep anesthesia at selected water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
7° C.:							
0 hour	5	9.50	127.2	41.92	21.4-72.0	7.13	--
6 hours	5	9.26	119.6	1.48	1.0- 2.2	0.21	0.58- 2.38
9 hours	5	9.82	135.8	1.00	0.8- 1.2	0.06	0.74- 1.26
24 hours	5	9.78	141.2	0.80	0.6- 1.0	0.06	0.54- 1.06
12° C.:							
0 hour	5	8.14	86.2	26.40	10.2-50.8	7.20	--
1 hour	5	9.64	129.4	9.72	7.4-17.2	1.92	0.46-17.98
3 hours	5	8.64	102.6	2.64	1.6- 4.4	0.48	0.57- 4.71
6 hours	5	8.94	116.0	1.04	0.8- 1.4	0.10	0.61- 1.47
9 hours	5	8.32	87.6	1.00	0.8- 1.4	0.14	0.60- 1.40
24 hours	5	8.76	95.8	1.44	1.0- 2.0	0.20	0.58- 2.30
17° C.:							
0 hour	5	10.22	167.4	18.04	9.4-23.8	2.58	--
6 hours	5	9.62	133.4	3.44	2.8- 3.8	0.17	2.71- 4.17
9 hours	5	9.82	143.8	1.52	0.8- 2.8	0.34	0.06- 2.98
24 hours	5	10.02	149.8	1.24	1.0- 1.6	0.11	0.77- 1.71

TABLE 7.--Residues of MS-222 including background (grams) in livers of rainbow trout following deep anesthesia at selected time intervals and water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error
		Length (inches)	Weight (grams)	Mean	Range	
[illegible] C.:						
0 hour	2	10.00	154.0	59.6	46.2-73.0	13.39
6 hours	2	9.70	131.5	17.5	15.8-18.2	1.20
9 hours	2	9.95	141.0	20.0	18.2-21.8	1.80
24 hours	2	10.00	146.5	15.0	14.8-15.2	0.06
12° C.:						
0 hour	2	9.85	141.5	60.6	57.2-64.0	3.40
1 hour	2	10.00	150.5	19.5	18.4-20.6	1.10
3 hours	2	9.80	150.0	17.9	16.0-19.8	1.90
6 hours	2	9.50	132.0	11.1	11.0-11.2	0.06
9 hours	2	9.25	118.0	13.4	12.0-14.8	1.39
17° C.:						
0 hour	2	10.55	187.0	49.3	31.0-67.6	18.30
6 hours	2	10.15	159.5	20.5	19.6-21.4	0.90
9 hours	2	10.25	166.5	17.5	17.0-18.0	0.50
24 hours	2	10.40	168.0	13.7	13.0-14.4	0.70

TABLE 8.--Residues of MS-222 including background (grams) in kidneys of rainbow trout following deep anesthesia at selected time intervals and water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error
		Length (inches	Weight (grams)	Mean	Range	
7° C.:						
0 hour	2	10.00	154.0	24.2	71.4-97.0	12.79
6 hours	2	9.70	131.5	9.4	9.2- 9.6	0.20
9 hours	2	9.95	141.0	6.6	6.0- 7.2	0.60
24 hours	2	10.00	146.5	6.4	6.0- 6.8	0.40
12° C.:						
0 hour	2	9.85	141.5	38.6	33.2-47.8	4.80
1 hour	2	10.00	150.5	15.5	11.6-19.4	3.90
3 hours	2	9.80	150.0	10.8	7.8-13.8	3.00
6 hours	2	9.50	132.0	8.7	8.4- 9.4	0.50
9 hours	2	9.25	118.0	9.1	7.0-11.2	2.10
[illegible]						
0 hour	2	10.55	187.0	62.8	58.8-66.8	4.00
6 hours	1	10.30	170.0	11.2	- -	-
9 hours	2	10.25	166.5	9.0	8.8-10.2	2.20
24 hours	2	10.40	168.0	5.4	5.2- 5.6	0.20

The reliability of the modified Bratton-Marshall colorimetric reaction for detection and measurement of MS-222 in liver and kidney is comromised by the high and variable concentrations of background amines. Therefore, residues in these tissues were measurable only at 0-hour withdrawal. The detection of residues in these organs is of little importance other than to indicate the ability of fish to deactivate or metabolize the anesthetic. The liver and kidney are rarely consumed by people, and they comprise only a small fraction of the total weight of a fish.

Brown trout.--Groups of 6- to 9-inch brown trout were brought to medullary collapse by 80 p.p.m. of MS-222 at 17° within 4.6 to 7.0 minutes. Because the species is less sensitive to the drug at 7° and 12°, 100-p.p.m. solution was used to achieve medullary collapse within 9.5 to 12.1 minutes at 7° and within 7.9 to 11.0 minutes at 12°.

The residues of MS-222 in brown trout were measured only in muscle (table 9). The concentrations, including background, ranged from 9.8 to 60.8 p.p.m. at 0-hour withdrawal and declined 80 to 99 percent within 6 hours. They were 0.4 to 5.4 p.p.m. at 6 hours and 0.6 to 1.4 p.p.m. at 24 hours. Also, they were least variable at 17° and persisted longer at 7°. After 24 hours, the mean residues of MS-222 were only a fraction of a part per million over background.

Brook trout.--Brook trout 7 to 10 inches long were anesthetized to medullary collapse. The concentrations of drug were 100 p.p.m. at 17°, 110 p.p.m. at 12°, and 120 p.p.m. at 7°. Anesthesia was induced within 4.2 to 5.4 minutes at 17°, 6.5 to 8.7 minutes at 12°, and 8.8 to 11.4 minutes at 7°.

The residues, including background, in muscle ranged from 6.0 to 34.8 p.p.m. at 0-hour withdrawal at the three temperatures (table 10). This range was lower than in any of the other trout, and it is noteworthy because the brook trout required higher concentrations of the drug for anesthesia. Within 9 to 24 hours after withdrawal, the residues including background were 0.6 to 2.8 p.p.m. At 24 hours, the mean residues of MS-222 exceeded the backgrounds by 1.1 p.p.m. at 17°, 0.3 p.p.m. at 12°, and 1.4 p.p.m. at 7°, but their 95-percent confidence intervals overlapped.

Lake trout.--Lake trout 6 to 8 inches long were brought to medullary collapse by 100 p.p.m. of MS-222 at 17°, 110 p.p.m. at 12°, and 135 p.p.m. at 7° within 3.9 to 6.5 minutes overall. The highest concentrations of residues, including background, were measured in muscle from fish at 7°. These fish had longer exposures to higher concentrations of drug than the others.

The mean values for residues of anesthetic at 6-, 9-, and 24-hour withdrawals at the

TABLE 9.--Residues of MS-222 including background in muscle of brown trout following deep anesthesia at selected time intervals and water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
[illegible]							
[illegible]	3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	--
6 hours	3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
9 hours	3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
24 hours	3	[illegible]	[illegible]	[illegible]	1.[illegible]	[illegible]	[illegible]
[illegible]							
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	20.8-[illegible].8	1.[illegible]	--
[illegible]	[illegible]	[illegible]	1.[illegible]	[illegible]	[illegible].[illegible]-[illegible].4	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	1.[illegible]	1.[illegible]- 2.0	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]- .8	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]- 1.4	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]- 1.0	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	--
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

TABLE 10.--Residues of MS-222 including background in muscle of brook trout following deep anesthesia at selected time intervals and water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
7° C.:							
0 hour	3	8.00	138.3	16.67	6.0-22.6	5.34	--
6 hours	3	8.83	123.0	3.53	3.2- 3.8	0.18	2.70-4.30
9 hours	3	9.00	118.3	2.60	2.4- 2.8	0.11	2.13-3.07
24 hours	3	8.43	94.7	1.93	1.8- 2.2	0.23	0.94-2.9
12° C.:							
0 hour	3	9.23	118.0	28.07	17.8-34.8	5.22	--
1 hour	3	8.77	110.3	3.73	3.2- 4.6	0.44	1.84- .
3 hours	3	9.63	148.3	1.37	0.7- 1.8	0.3	. - .
6 hours	3	8.20	81.7	0.83	0.8- 0.9	0.03	. - .
9 hours	3	8.70	106.7	1.03	0.9- 1.2	0.10	. - .
24 hours	3	8.60	90.7	0.80	0.6- 1.0	0.11	. - .
17° C.:							
0 hour	3	8.93	112.0	15.47	13.0-19.0	1.86	--
6 hours	3	9.73	143.0	3.33	3.0- 3.6	0.18	. - .
9 hours	3	8.33	110.0	2.33	2.2- 2.4	0.07	. - .
24 hours	3	8.83	102.0	1.67	1.2- 2.0	0.24	. - .

TABLE 11.--Residues of MS-222 including background in muscle of lake trout following deep anesthesia at selected time intervals and water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
7° C.:							
0 hour	3	7.43	46.3	31.00	20.0-55.6	11.83	--
6 hours	3	7.17	42.3	3.40	2.8- 4.4	0.50	1.25-5.
9 hours	3	7.33	43.3	4.53	3.8- 5.6	0.54	2.21-6.8
24 hours	3	6.73	35.3	2.87	2.6- 3.2	0.18	2.10-3.
12° C.:							
0 hour	3	7.13	43.3	31.67	22.8-37.4	4.50	--
1 hour	3	7.30	40.7	6.80	6.0- 8.2	0.70	3.
3 hours	3	6.67	30.7	1.00	0.8- 1.2	0.11	0.
6 hours		7.73	51.7	0.93	0.8- 1.0	0.07	0.
9 hours	3	7.18	40.3	1.40	1.0- 1.8	0.23	0.
24 hours	3	6.93	45.0	0.87	0.8- 1.0	0.05	0.
17° C.:							
0 hour	3	7.83	53.3	14.87	14.0-16.6	0.96	--
6 hours	3	7.57	48.7	2.27	2.0- 2.6	0.18	1.50-3.04
9 hours	3	7.40	41.7	1.80	1.6- 2.0	0.11	1.33-3.27
24 hours	3	7.46	43.7	1.40	1.2- 1.6	0.11	0.93-1.87

three temperatures were 0 to 4 p.p.m. above backgrounds, but the overlap of confidence intervals suggests that little, if any, residue remains beyond 9 hours (table 11). The residues dissipated more rapidly at 12°, and no significant amount persisted after 3 hours. The dissipation was intermediate and gradual at 17° and slowest at 7°.

DISCUSSION

Residues of MS-222 dissipated rapidly in rainbow trout, brown trout, brook trout, and lake trout within 1 to 6 hours after withdrawal from deep anesthesia (fig. 2). Regardless of species and temperature, there was very little difference in muscle between the residues of MS-222 and quantities of background amines at 9 to 24 hours. The higher concentrations of residues in all fish were measured at 0-hour withdrawal, and it was here that differences were greater among individuals and species.

Rainbow trout and brown trout had higher initial residues than brook trout and lake trout despite the fact that the latter species were exposed to higher concentrations of MS-222. The chars, however, had the briefer exposures.

The chars may have oral and gill membranes which are less permeable than those of trouts to MS-222. The lake trout appear to be less able than brook trout to metabolize or detoxify the drug because they showed less tolerance and higher residues. Also, the anesthetic is more toxic to lake trout than brook trout (Marking, 1966).

The mineral content of water affected the mean time to anesthetize fish and the amount of residue in them. The 0-hour residues in

fish exposed in soft water (10 p.p.m. total hardness) were triple those in fish in hard water (180 p.p.m. total hardness). The fish in soft water, however, were exposed twice as long as in harder water. At the end of 24 hours, there were no significant differences in residues.

Acetylated residues in fish were not unexpected when one considers the similarity of MS-222 to p-aminobenzoic acid and the sulfonamides. These aromatic amines were probably acetylated in the liver or kidney and excreted (Hawk et al., 1954; Jessop, 1961). We do not know whether the conjugation of MS-222 with acetic acid in fish is a significant mechanism of detoxification or deactivation.

In general, the residues of MS-222 in fish at 7° and 12° were greater at 0-hour withdrawal than at 17° (figure 2). They persisted longer at 7°, but the concentrations of anesthetic solution and the durations of anesthesia also were greater at this temperature. The shorter exposures to lower concentrations of drug at 17° resulted in less but more consistent residues in all species at 0-hour withdrawal.

CONCLUSIONS

Residues of MS-222 occurred in the blood, muscle, liver, and kidney of deeply anesthetized rainbow trout. They dissipated rapidly within 1 to 6 hours, but those in liver and kidney were masked by background substances which interfered with the modified Bratton-Marshall colorimetric method.

The residues of MS-222 in muscle varied greatly at 0-hour withdrawal among anesthetized rainbow trout, brown trout, brook trout, and lake trout. They were higher in the trouts than in the chars. Regardless of species and temperature, there was very little difference between the residues of MS-222 and background amines in muscle at 9 to 24 hours after withdrawal. The residues of anesthetic in muscle of fish at 7° and 12° C. were greater at 0-hour withdrawal than at 17°. Residues in muscle persisted longer at 7° than at warmer temperatures.

The 0-hour residues of drug in muscle of fish exposed in soft water were triple those of fish in hard water. At the end of 24 hours, however, there were no significant differences in residues.

SUMMARY

The residues of MS-222 in selected tissues of rainbow trout, brown trout, brook trout, and lake trout at 7°, 12°, and 17° C. and in waters of various hardnesses were measured by means of the modified Bratton-Marshall colorimetric method. The concentrations of drug in the blood, muscle, liver, and kidney of deeply anesthetized rainbow trout dissipated rapidly within 1 to 6 hours. Those in the liver and kidney were masked by background substances which interfered with the colorimetric method.

The amounts of MS-222 including background in muscle of the four species at 0-hour withdrawal were 6 to 72 p.p.m. The mean concentrations at 7°, 12°, and 17° C. were 18 to 42 p.p.m. in rainbow trout, 13 to 44 p.p.m. in brown trout, 15 to 28 p.p.m. in brook trout, and 15 to 32 p.p.m. in lake trout. At 24 hours after withdrawal from the anesthetic, the mean concentrations including background were 0.8 to 1.2 p.p.m. in rainbows, 0.8 to 1.4 in browns, 0.8 to 1.9 in brook trout, and 0.9 to 2.9 p.p.m. in lake trout. Actually, there was little difference between the residues of drug and background amines at 9 to 24 hours after withdrawal.

At 7° and 12° the concentrations of residue including background were generally above 20 p.p.m. at 0-hour withdrawal, whereas at 17° they were below 20 p.p.m. The residues persisted longer at 7° than at the warmer temperatures. At 9 to 24 hours the concentrations of drug at the three temperatures approached those of background amines in controls.

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Davis, H. S.
1956. Culture and diseases of game fishes. University of California Press, Berkeley and Los Angeles. 332 p.

Hawk, Phillip B., Bernard L. Oser, and William H. Summerson.
1954. Practical physiological chemistry. 13th ed. McGraw-Hill Book Co., New York. 1439 p.

Jessop, William John Edward.
1961. Fearon's introduction to biochemistry. 4th ed. Academic Press, New York. 473 p.

Lennon, Robert E., and Charles R. Walker.
1964. Investigations in Fish Control: 1. Laboratories and methods for screening fish-control chemicals. U.S. Bureau of Sport Fisheries and Wildlife, Circular 185. 15 p.

Marking, Leif L.
1966. Investigations in Fish Control. 12. The toxicity of MS-222 to selected fishes: U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 18.

Schoettger, Richard A.
1966. Investigations in Fish Control: 16. Annotated bibliography on MS-222. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 22.

Schoettger, Richard A., and Arnold M. Julin.
1966. Investigations in Fish Control: 13. Efficacy of MS-222 as an anesthetic on four salmonids. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 19.

Walker, Charles R., and Richard A. Schoettger.
1966. Investigations in Fish Control: 14. Method for determining MS-222 residues in fish. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 20.

16. Annotated Bibliography on MS-222

By Richard A. Schoettger

United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
Resource Publication 22 • January 1967 • Washington, D.C.

ANNOTATED BIBLIOGRAPHY ON MS-222

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Abstract.--This bibliography contains 86 selected references on uses of MS-222 on cold-blooded animals including fish and amphibians. Most of the references are annotated.

The Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., initiated studies in 1964 on the toxicity, efficacy, and residues of MS-222 in fish. The data were required by the U.S. Food and Drug Administration in order to clear the drug for continued use. In connection with the studies, a substantial bibliography on uses of MS-222 was compiled. A considerable number of the more useful references were annotated.

Some recognized references on MS-222 were not available conveniently for review and they have been included by title only.

BIBLIOGRAPHY

Allison, Leonard N.

1961. The effect of tricaine methanesulfonate (M.S. 222) on motility of brook trout sperm. Progressive Fish-Culturist, vol. 23, no. 1, p. 46-48.

The spermatozoa of brook trout remained motile less than 10 seconds in a concentration of 18.9 p.p.m. of MS-222. Allison recommended that the drug should not contact reproductive products during spawn-taking.

Bailey, Merryll M.

1965. Lake trout fin-clipping rates at two national fish hatcheries. Progressive Fish-Culturist, vol. 27, no. 3, p. 169-170.

MS-222 was used as an anesthetic during the fin-clipping of more than 3.5 million lake trout. The fish ranged from 4.1 to 7.9 inches long and were young-of-the-year to 3-year-olds. One fin each was removed from 2 million fish, and 2 fins each were clipped on 1.5 million. Postmarking mortality usually was 0.1 to 0.2 percent; it increased with temperatures over 65° F. Cooling the solutions and reducing the concentration of MS-222 lowered mortality.

Ball, J. N., and P. N. Cowen.

1959. Urethane as a carcinogen and as an anaesthetic for fishes. Nature, vol. 184, p. 370.

Urethane is carcinogenic in the lung and other tissues of mice and rats. It does not produce tumors in rabbits, chickens, and guinea-pigs, but carcinogencity may vary with species (and strain). It has a leucopenic effect on humans and can be absorbed from the skin.

MS-222 has not been reported to be carcinogenic. Its effectiveness on fish and amphibians varies with species, size of the animal, and temperature. Suitable concentrations are determined empirically for every species and situation.

Baudin, Louis.

1932a. Action de la tricaïne sur la consommation d'oxygène de Carassius auratus. Comptes rendus des Séances de la Société de biologie, vol. 109, p. 731-733.

A 1:1,000 solution of MS-222 anesthetized goldfish within 1 to 2 minutes. Observations

on the circulation of blood in the fins revealed a slower than normal rate with prolonged exposure to the anesthetic. A concentration of 1:20,000 produced a level of anesthesia in 30 to 60 minutes at 16.5° C. which could be maintained for 24 hours without apparent damage. The rate of oxygen consumption was depressed to 50 percent of normal after 1 hour and to 25 percent after 11 hours of exposure. The respiratory rate returned to normal within 2 hours after the fish were placed in fresh water. Above 16° C., narcosis was incomplete and the reduction of oxygen consumption was less. Below this temperature, a longer period was required for anesthesia and, for a fixed period, the rate of oxygen consumption was higher than at 16.5° C.

1932b. Action de la tricaïne sur le quotient resperatoire de Carassius auratus. Ibid., p. 1081-1083.

1932c. Perte de la sensibilité a la dépression chez les poissons anesthésiés a la tricaïne. Ibid., vol. 110, p. 151-153.

Oxygen consumption by goldfish was increased at lower atmospheric pressures. The rates for fish which were anesthetized with 1:20,000 of MS-222 were relatively low under conditions of both normal and reduced pressure. The sensitivity of the fish to reduced pressure was not completely suppressed by concentrations of 1:30,000 and 1:40,000.

1932d. Respiration du poisson (Carassius auratus) anesthésié à la tricaïne et soumes à une élévation brusque de température. Ibid., p. 235-237.

1934. Action de la tricaïne sur le sang des poissons. Ibid., vol. 115, p. 510-512.

A 1-percent solution of MS-222 anesthetized several species of fish, including goldfish and perch, within 1 minute. There were no effects on the erythrocytes, erythrocyte count, or oxygen capacity of the blood. The oxygen saturation of the blood was near zero, and the carbon dioxide level was slightly in excess. Lower concentrations of the drug and longer exposures induced exaggerated respiratory movement which increased the oxygen saturation and lowered the carbon dioxide level. The author concluded that MS-222 may excite respiratory centers of the brain, or interfere with exchanges of gases, thereby causing anoxia and hyperventilation. With complete narcosis, the blood showed signs of asphyxia and there was a slowing of the circulation which contributed to reductions in erythocyte numbers and oxygen capacity of the blood.

Bell, Gordon R.

1964. A guide to the properties, characteristics, and uses of some general anaesthetics for fish. Fisheries Research Board of Canada, Bulletin 148, 4 p.

Properties of eleven chemicals used as fish anesthetics are summarized. The chemicals are carbon dioxide, chloral hydrate, chloretone, ether, methyl pentynol, MS-222, phenoxyethanol, quinaldine, sodium amytal, tribromoethanol, and tertiary amyl alcohol. The common and chemical names of the chemicals, manufacturers, costs, solubilities, stabilities, hazards, toxicities, emergency treatments, approximate dosages, precautions, behavioral effects, uses, and modes of action are included. Concentrations of MS-222 from 1:9,000 to 1:4,500 were recommended for brief exposures. The generally useful range is between 1:25,000 to 1:12,500.

Black, Edgar C., and Anne R. Connor.

1964. Effects of MS 222 on glycogen and lactate levels in rainbow trout (Salmo gairdneri). Journal of the Fisheries Research Board of Canada, vol. 21, no. 6, p. 1539-1542.

A concentration of 0.5 g. of MS-222 per gal. anesthetized trout within 60 seconds. The fish showed signs of initial stimulation. The dosage might have been fatal with prolonged exposure. The hemoglobin, blood and muscle lactate, and muscle glycogen levels were similar in anesthetized and control fish.

Blahm, T. H.

1961. Effect of tricaine methanesulfonate on oxygen consumption of juvenile sockeye salmon. Transactions of the American Fisheries Society, vol. 90, no. 2, p. 226-227.

MS-222 uniformly reduced the oxygen consumption of both large (160-180 mm. fork

length) and small (60-80 mm. fork length) sockeye salmon. The fish were anesthetized by 1:20,000 in a constant flow respirometer.

Bové, Frank J.

1962. MS-222 Sandoz--the anaesthetic of choice for fish and other cold-blooded organisms. Sandoz News, no. 3, 12 p.

The author summarizes the history of MS-222 from its use as a local anesthetic in human medicine to its current status as a general anesthetic for fish, amphibians, and other cold-blooded forms. His personal communications with various investigators reveal that concentrations ranging from 1:3,785 to 1:17,500 have been used in measuring and weighing, marking, and spawning of various salmonids. A concentration of 1:12,000 seemed to be most popular. Solutions containing approximately 1:3,000 of MS-222 were effective on tropical fish, goldfish, bluegills, largemouth bass, and bullheads.

Effective dosages for amphibians ranged from 1:250 to 1:20,000, but 1:1,000 to 1:3,000 were preferred. Specimens with gills and those undergoing metamorphosis were more sensitive to the anesthetic.

Butler, Robert L.

1957. The development of a vinyl plastic subcutaneous tag for trout. California Fish and Game, vol. 43, no. 3, p. 201-212.

MS-222 at 0.5 g./gal. prepared fish for the operation within 30 seconds at 50° F. The fish were exposed no longer than 3 minutes. As temperature increases, it is necessary to reduce the exposure time or the concentration. The drug had no effect on feeding by the fish after they recovered from anesthetization.

Campbell, G. D., and D. H. Davies.

1963. Effect of ethyl m-aminobenzoate (MS-222) on the elasmobranch electrocardiograph. Nature, vol. 198, no. 4877, p. 302.

MS-222 caused coincidental decreases in the pulse and respiratory rates of stingrays indicating a neurological relationship between the cardiac and respiratory centers in the brain. The drug did not affect the ECG complex.

Christensen, K.

1931. Effect of castration on the secondary sex characters of males and females of Rana pipiens. Anatomical Record, vol. 48, p. 241.

Collins, James L., and Andrew H. Hulsey.

1963. Hauling mortality of threadfin shad reduced with M.S. 222 and salt. Progressive Fish-Culturist, vol. 25, no. 2, p. 105-106.

A combination of 0.5-percent salt and MS-222 equivalent to 1 g./12 gal. facilitated hauling of more than 200,000 fish with a survival of 95 percent.

Copenhaver, W. M.

1939. Initiation of beat and intrinsic contraction rates in the different parts of the Amblystoma heart. Experimental Zoology, vol. 80, p. 139.

Crawford, Bruce, and Andrew Hulsey.

1963. Effects of M.S. 222 on the spawning of channel catfish. Progressive Fish-Culturist, vol. 25, no. 4, p. 214.

The anesthetization of adult channel catfish with MS-222 at a rate of 4 g./8 gal. did not affect the success of spawning nor the viability of fry. The fish were narcotized for sexing and placement in spawning pens.

Dollar, Alexander M.

1963. Air transportation of living rainbow trout. Progressive Fish-Culturist, vol. 25, no. 3, p. 167-168.

The fish were placed in lake water containing MS-222 at a concentration of 1:100,000. The water was cooled gradually over a 3-hour period at 33° F. The fish and water were transferred to a plastic bag and packed in ice. Oxygen was added to the bags. The fish survived in the sealed containers for 24 hours.

Eisler, Ronald, and Tadeusz Backiel.

1960. Narcotization of chinook salmon fingerlings with tricaine methanesulfonate (M.S. 222). Transactions of the American Fisheries Society, vol. 89, no. 2, p. 164-167.

A concentration of 1:33,000 effectively anesthetized this species within 5 minutes.

The rate of narcotization was dependent on concentration. Recovery time increased with exposures up to an hour, but declined with longer exposure. The drug was equally active in fresh and salt water. The authors reviewed concentrations of MS-222 which have been used to anesthetize various species of fish.

Ellis, Robert J.

1964. The effect of confinement on blood lactate levels in chinook and coho salmon. Research Briefs, Fish Commission of Oregon, vol. 10, no. 1, p. 28-34

The lactic acid levels in the blood of troll-caught salmon declined more rapidly in those individuals which were held in solutions containing MS-222 at a concentration of 1:150,000.

Ewing, Ann.

1965. Current U.S. patents. A method for marking fish by which scales are transplanted painlessly from one part of the body to another has been patented. Science News Letter, vol. 87, no. 15, p. 228.

Dr. Louis Levy and Miss Carol A. De Fusco (1965) have patented a method of marking fish by replacing scales of one color from one part of the body with those of contrasting color from a different area. The fish are anesthetized during the operation with 50 to 100 p.p.m. of MS-222.

The technique was successful on goldfish, carp, blue acara, blackspot barb, and guppies, and has been used to measure the effects of drugs on the rejection time of scales from different fish.

Friddle, S. B., and S. F. Snieszko.

1950. Effect of tricaine methanesulfonate on the determination of sulfonamides. Science, vol. 112, no. 2902, p. 181-182.

Concentrations of 2 to 4 mg.%, as sulfamerazine, were detected in the tissues of trout which should not have contained the sulfa drug. The fish had been anesthetized for 1 minute in a 1:5,000 solution of MS-222. This anesthetic, or others with similar molecular structures, should not be used whenever they may interfere with the colorimetric test for sulfonamides.

Fromm, Paul O.

1958. A method for measuring the oxygen consumption of fish. Progressive Fish-Culturist, vol. 20, no. 3, p. 137-139.

MS-222 is used to immobilize fish for length and weight measurements before they are placed in a respirometer. A concentration of 0.03 percent produces anesthesia within 30 to 45 seconds. The drug apparently has no lasting effect on the general metabolic rate.

Gebhards, Stacy V.

1965. Transport of juvenile trout in sealed containers. Progressive Fish-Culturist, vol. 27, no. 1, p. 31-36.

The use of sedating levels of MS-222 did not increase the loading density or survival of rainbow trout in sealed containers. Greater survival was associated with the starvation period prior to loading.

Gilbert, P. W., and F. G. Wood.

1957. Methods of anaesthetizing large sharks and rays safely and rapidly. Science, vol. 126, p. 212.

A 1:1,000 solution of MS-222 is sprayed into mouth, spiracles, or gill exits by means of a hand sprayer, such as a water pistol. Sharks as large as 400 pounds are anesthetized in a minute or less and may be handled, out of water, for 5 to 30 minutes. The volume of anesthetic solution required to anesthetize fish varies with the size of the individuals.

Glücksohn, Salome.

1932. Äussere Entwicklung der Extremitäten und Stadieneinteilung der Larvenperiode von Triton taeniatus Leyd. und Triton cristatus Laur. W. Roux' Archiv fur Entwicklungsmechanik der Organismen, vol. 125, p. 341-405.

Metamorphosing salamanders were anesthetized with 1:3,000 of MS-222 for 30 minutes a day. The growth of treated specimens was somewhat less than controls, but the former were proportioned normally.

Goodrich, H. B., and R. Nichols.

1931. The development and regeneration of color pattern in Brachydanio rerio. Anatomical Record, vol. 51, p. 513.

Gossington, Robert.
1957. An aid to fish handling--tricaine. Aquarium Journal, vol. 28, no. 9, p. 318-321.

Fish could be anesthetized safely with MS-222 in concentrations of 0.24 to 0.32 g./gal. Larger specimens required somewhat higher concentrations. Live-bearers were generally more resistant than egg-layers. The drug reduced the chances of injury to fish from thrashing during shipment. Under anesthesia, Siamese fighting fish could be shipped together in a single container.

Hadian, Z., and M. S. Dunn.
1938. Localisation in the occulomotor nuclei of the goldfish. Journal of Comparative Neurology, vol. 68, p. 191.

Hublou, Wallace F.
1957. A method of using an anesthetic in marking fins. Progressive Fish-Culturist, vol. 19, no. 1, p. 40-43.

MS-222 was used in a recirculating system to fin-clip fingerling salmon and steelhead trout. The marking rate was improved by 49.5 percent with the use of an anesthetic. Advantages of the system included better marks, lower rate of injury, reduction of worker fatigue, and saving in time and money.

Johnson, Harlan E., and J. M. Shelton.
1958. Marking chinook salmon fry. Progressive Fish-Culturist, vol. 20, no. 4, p. 183-185.

Groups of 10 to 20 individuals were caught in a small net and placed in a 1:7,500 solution of MS-222. Fin-clipping began when all the fish were on their sides and after the net had been placed in fresh water. The last fish was marked just before it recovered. Approximately 487,000 fry were marked at a cost of $50.

Karczmar, Alexander G., and Theodore Koppanyi.
1948. Action of central nervous system depressants at different growth periods of salamander (Amblystoma punctatum) larvae. Federation Proceedings, vol. 7, p. 231-232.

Larvae of different ages were immersed in a 1:7,500 solution of MS-222, and the times for anesthesia were recorded. Paraldehyde, ethyl alcohol, sodium barbital, nembutal, chloral hydrate, and chloretone were also tested. Anesthesia with MS-222 was more rapid in older and larger individuals.

Klontz, George W.
1964. Anesthesia of fishes. From: Proceedings of the Symposium on Experimental Animal Anesthesiology, Brooks Air Force Base, December 14-16, 13 p.

The efficacy and characteristics of 14 methods which are used to anesthetize fish were discussed.

Concentrations of 25 to 35 p.p.m. of MS-222 are recommended for transporting fish; 50 to 100 p.p.m. are used to induce deep anesthesia. In general, induction time requires 1 to 3 minutes of exposure and the fish recover, in fresh water, within 3 to 15 minutes. Fish which are repeatedly exposed to MS-222 showed a slight increase in tolerance which is corrected by raising the concentration slightly. The drug appears to be toxic to those fish which are treated in salt water and in direct sunlight.

Knight, Alexis E.
1964. Intracellular hemoglobin crystallization in two centrarchids, the largemouth bass and the bluegill. Progressive Fish-Culturist, vol. 26, no. 3, p. 115-117.

A 1:5,000 solution of MS-222 was used to narcotize fish during the collection of blood samples.

Koppanyi, Theodore, and Alexander G. Karczmar.
1948. Comparison of anesthetic action of acetanilid, tricaine (MS-222) and aliphatic depressants. Federation Proceedings, vol. 17, p. 234.

The action of acetanilid on salamander larvae was independent of larval stage. Subanesthetic dosages of the drug acted additively with subanesthetic levels of MS-222, nembutal, and chloretone. The anesthetic effects of chloretone, MS-222, alcohol, paraldehyde, and acetanilid were reversed rapidly.

Lemarque, Pierre.
1964. Anesthesie et transport. Bull. Inf. Cons. Sup. Pêche, vol. 55, p. 5-9.

MS-222 may be used in concentrations of 1:10,000 to 1:50,000 to anesthetize fish before they are placed in plastic bags at a loading level of 1 kilogram of fish per 1 to 2 liters of water. The bags were filled with oxygen. Dosages of 1:100,000 were recommended for the tranquilization of fish in transportation tanks.

Larsen, Howard N.
1964. Comparison of various methods of hemoglobin determination of catfish blood. Progressive Fish-Culturist, vol. 26, no. 1, p. 11-15.

The fish were anesthetized in a 1:5,000 solution of MS-222 to facilitate the collection of blood samples.

Levy, Louis Encino, and Carol A. DeFusco.
1965. Identification of scaly teleosts. U.S. Patent Office, Patent No. 3,174,458. 3 p.

Lumb, William V.
1963. Small animal anesthesia. Chapter: Anesthesia of laboratory and zoo animals, p. 269-310. Lea and Febiger, Philadelphia. 420 p.

MS-222 is used to immobilize fish and other cold-blooded animals by completely bathing small subjects, by gill spraying in large fish, or by injection in large animals. Concentrations of 0.5 to 1.0 grams of drug per gallon are used for most teleosts and the temperature is maintained at 40° to 60° F. Repeated use of anesthetic solutions reduces their efficacy. Longer anesthesia or sedation can be maintained with lower concentrations. The drug can also be used in treatment of fungus infections and other localized diseases on pet or ornamental fish.

The uses of ether, sodium amytal, carbon dioxide, urethane, and cresol in anesthetizing fish are discussed.

Marking, Leif L.
1966. Investigations in Fish Control. 12. Toxicity of MS-222 to selected fishes. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 18.

The 24-hour LC_{50} concentrations of MS-222 for various species of fish, at 12° C., were found to be: rainbow trout, 39.0 to 52.0 p.p.m.; brown trout, 38.5 to 45.6 p.p.m.; brook trout, 50.7 to 52.2 p.p.m.; lake trout, 33.8 to 39.8 p.p.m.; northern pike, 56.0 p.p.m.; bluegill, 45.7 to 46.9 p.p.m.; largemouth bass, 42.0 to 61.5 p.p.m.; and walleye, 49.0 p.p.m. The results indicated that exposures of MS-222 for 24 to 96 hours had no significant effect on the toxicity of the chemical.

In general, MS-222 was more toxic to smaller individuals, and at higher temperatures. Water hardness had little effect on toxicity.

The safety index of MS-222 for rainbow trout was determined by comparisons of the LC_{50} and EC_{50} concentrations after 15, 30, and 60 minutes of exposure in relatively soft and hard water. The indexes ranged from 1.7 to 2.0 and decreased slightly with exposure time. A comparison of the LC_1 and EC_{99} for hard water gave an index of about 1.3 and 1.0 to 1.1 in soft water.

Maintenance of anesthesia in fish for 96 hours was not harmful. After the fish had been placed in fresh water and recovered, they fed as well as controls.

Martin, N. V., and D. C. Scott.
1959. Use of tricaine methanesulfonate (M.S. 222) in the transport of live fish without water. Progressive Fish-Culturist, vol. 21, no. 4, p. 183-184.

Hybrid trout were anesthetized in 60 p.p.m. of MS-222 and packed in layers of chipped ice and sphagnum moss. The fish were maintained under these conditions for 4 to 4.5 hours with little mortality.

McFarland, William N.
1959. A study of the effects of anesthetics on the behavior and physiology of fishes. Publications of the Institute of Marine Science, University of Texas, vol. 6, p. 23-55.

The anesthetic effects of 21 chemicals, including MS-222, were tested against Fundulus parvipinnis, Gambusia affinis, Paralabrax clathratus, and Girella nigricans. The behavioral changes induced in fish by anesthetics were classified into the following levels of anesthesia: sedation, loss of

equilibrium, loss of reflex reactivity and medullary collapse. Anesthesia in fish was compared to that in humans and was found to be a similar process involving sequential suppression of higher to lower central nervous centers.

The narcotic potencies of the various compounds increased with their molecular weights. MS-222 was rated as highly potent. The ratio of the dosages necessary to induce sedation and medullary collapse during a 12-hour period was 7.1.

Anesthesia with MS-222 was more rapid at 27° than at 12° C., but anesthesia did not progress as deeply at the lower temperature. Metabolic studies indicated that MS-222 was depleted, with time, at a greater rate than other anesthetics.

McFarland, William N.
1960. The use of anesthetics for the handling and the transport of fishes. California Fish and Game, vol. 46, no. 4, p. 407-431.

MS-222, tertiary amyl alcohol, and methylparafynol were suggested as beneficial for the induction of deep anesthesia because they act quickly and recovery is rapid. Recovery from anesthesia was complete provided respiratory movements had not ceased for more than a few minutes. MS-222 at 0.03 g./gal. induced loss of reflex in Fundulus parvipinnis within 1 hour. Higher concentrations were recommended for more rapid anesthesia; however, fish must be removed from the anesthetic after the desired stage of anesthesia has been induced.

MS-222 was not recommended for transporting fish. The drug failed to maintain a lowered rate of metabolism at higher temperatures.

It is advisable to pretreat fish in an anesthetic before transporting to reduce metabolic rates which may be stimulated due to handling.

McGovern, Beulah H., and Roberts Rugh.
1944. Efficacy of m-amino ethyl benzoate as an anesthetic for amphibian embryos. Proceedings of the Society for Experimental Biology and Medicine, vol. 57, p. 127-130.

Dosages of 1:3,000 did not affect the motility or fertility of frog spermatozoa. The eggs which were fertilized in this solution developed normally when the exposure did not exceed 1 hour. Longer exposures produced decreasing numbers of abnormal embryos as development progressed through gastrulation and neurulation. The older embryos withstood anesthesia for 24 hours; however, mortality of the embryos undergoing transition from external to internal gill respiration increased with immersions in MS-222 longer than 2 hours. The drug inhibited muscular action, but not ciliary activity. MS-222 was considered to be nontoxic to frog embryos within the exposure times adequate for surgical operations.

Meehan, William R., and L. Revet.
1962. The effect of tricaine methanesulfonate (M.S. 222) and/or chilled water on oxygen consumption of sockeye salmon fry. Progressive Fish-Culturist, vol. 24, no. 4, p. 185-187.

The most favorable conditions for survival of fish during transportation appeared to be uncrowded numbers of fish in water colder than that from which they were removed. The fish also survived well when uncrowded in their normal environmental water to which was added 0.1 g. of MS-222 per 4,000 ml. Unsatisfactory results were obtained with crowding, or when the fish were placed in solutions of MS-222 which were colder than their environmental water.

Meister, Alfred L., and Charles F. Ritzi.
1958. Effect of chloretone and MS-222 on eastern brook trout. Progressive Fish-Culturist, vol. 20, no. 3, p. 104-110.

MS-222 was considered superior to chloretone as an anesthetic for fishery use. The former had a wider range of practical field concentrations, lesser inhibitory effect on respiration, and was easier and more predictable for use in the field. Both drugs produced more rapid anesthetization when temperatures were increased. Anesthesia with MS-222 was induced within approximately 10 minutes by concentrations ranging from 1:5,000 to 1:15,000 at 37° to 39° F., and 1:5,000 to 1:25,000 at 48° to 51° F. A concentration of 1:1,000 produced respiratory

arrest in brook trout after 5 minutes, but continued exposure for 6 minutes was not fatal.

They observed that 22 to 35 pounds of brook trout, 36 to 86 pounds of salmon or 93 pounds of lake trout could be anesthetized per gram of MS-222.

Moss, D. D., and D. C. Scott.

1964. Respiratory metabolism of fat and lean channel catfish. Progressive Fish-Culturist, vol. 26, no. 1, p. 16-20.

MS-222 was used at the rate of 1 g./3.8 l. to anesthetize channel catfish for measurements of lengths and weights. The fish were placed in a respirometer and recovered from the anesthetic within 1 to 2 minutes. The oxygen consumption of fat fish was greater than that of thin fish at 25° C. At 30° C. the respiratory rates for both groups were similar.

Nelson, P. R.

1953. Use of three anesthetics on juvenile salmon and trout. Progressive Fish-Culturist, vol. 15, no. 2, p. 74.

MS-222, chlorobutanol, and urethane were used as aids in weighing and measuring coho salmon, red salmon, and Dolly Varden trout. MS-222 was used at the rate of 1:12,500 at 12° to 17° C. and effectively anesthetized the fish within several minutes. The concentration was increased slightly for fingerlings. A dosage of 1:10,000 caused 100 percent mortality.

Normandeau, Donald A.

1962. Microhematocrit values for some salmonids reared in New Hampshire. Progressive Fish-Culturist, vol. 24, no. 4, p. 172-176.

A solution containing 1:10,000 of MS-222 was used to anesthetize landlocked salmon, rainbow, brook, lake, and splake trout before collection of microhematocrits. The fish were anesthetized sufficiently within 1 minute. There was a significant relation of mean hematocrits to sampling date, but not to water temperature.

Parkhurst, Z. E., and M. A. Smith.

1957. Various drugs as aids in spawning rainbow trout. Progressive Fish-Culturist, vol. 19, no. 1, p. 39.

MS-222, sodium amytal, methyl pentynol, urethane, and chloretone were used to anesthetize rainbow trout.

A concentration of 264 p.p.m. of MS-222 induced complete anesthesia within 30 to 45 seconds. Longer exposures resulted in some mortality. The fish were in good condition 75 days after treatment. The hatching success of eggs from anesthetized and control fish was similar.

Methyl pentynol at 2,400 p.p.m. was effective within 3.5 minutes, a 0.5-percent solution of urethane in 2 minutes, and 400 p.p.m. of choretone in 1.0 to 1.5 minutes. Sodium amytal, because of its slow action, had no practical value.

The experiments were conducted at a temperature of 43° F.

Phillips, Arthur M., Jr., Henry A. Podoliak, Donald R. Brockway, and Ray R. Vaughn.

1957. The nutrition of trout. Cortland Hatchery Report 26, New York Conservation Department, Fisheries Research Bulletin 21. 93 p.

The absorption of radioactive cobalt was elevated in brook trout which were narcotized with MS-222. It was suggested that there may be an adjustment of the osmotic processes of narcotized fish.

Pickford, Grace E.

1953. A study of the hypophysectomized male killifish, Fundulus heteroclitus (Linn.) Bulletin of the Bingham Oceanographic College, vol. 14, no. 2, p. 5-41.

1957. Methods of hypophysectomy in fishes. Appendix to: The physiology of the pituitary gland of fishes, by Grace E. Pickford and James W. Atz, p. 485-487. New York Zoological Society, New York.

MS-222 is recommended over several other techniques and agents for anesthetizing fish during hypophysectomy. The drug gave excellent results with Fundulus heteroclitus, but suitable strengths must be determined for each species.

Piper, Robert G., and Robert F. Stephens.

1962. A comparative study of the blood of wild and hatchery reared lake trout.

Progressive Fish-Culturist, vol. 24, no. 2, p. 81-84.

Blood samples were collected by heart puncture after anesthetizing the trout in a 1:1,000 solution of MS-222. The hemoglobin levels and erythrocyte counts of the hatchery-reared and wild lake trout were similar.

Pulford, Earl F., and L. M. Woodall.
1963. An operculum marking experiment on juvenile chinook salmon. Research Briefs, Fish Commission of Oregon, vol. 9, no. 1, p. 30-36.

MS-222 was used as an anesthetic during marking of 1.5-inch salmon. There was little mortality of the fish which were maintained under observation for 112 days.

Randall, D. J.
1962. Effect of an anaesthetic on the heart and respiration of telost fish. Nature, vol. 195, no. 4840, p. 506.

Heart and respiratory rates were measured in tench exposed to 25 to 200 mg./l. of MS-222 at 17° C. The heart rate in undisturbed, control fish was 15 to 30 beats per minute. At a concentration of 33 p.p.m. of MS-222, the rate exceeded 50 per minute, and increased at higher dosages. The respiratory rate and amplitude also increased, but in a variable manner.

MS-222 probably acts on the heart via the parasympathetic nervous system since the direct effect of the drug on isolated and perfused hearts of tench, trout and roach decreased the beat frequency. Bilateral sectioning of the vagi of the fish, which were exposed to MS-222, resulted in a reduced heart rate.

Respiratory collapse occurred at 100 to 200 p.p.m. of MS-222.

Robinson, Clay.
1965. Those chasing-rainbows. U.S. Trout News, January-February, p. 5.

The anesthetic action of MS-222 varies according to water temperature and hardness.

Robertson, O. H.
1958. Accelerated development of testis after unilateral gonadectomy, with observations on normal testis of rainbow trout. U.S. Fish and Wildlife Service, Fishery Bulletin, No. 127, vol. 158, p. 9-30.

MS-222 was used to anesthetize fish for gonadectomy and for length and weight measurements. A concentration of 1:20,000 induced adequate anesthesia in 2 to 3 minutes. The fish recovered rapidly, and no harmful effects were observed even after daily use for a number of weeks.

The operational technique included starvation of the fish for 48 hours and initial anesthesia in 1:20,000 of MS-222. The fish were placed on an operating board and then dipped into a 1:25,000 solution of the anesthetic.

The sequence of histological changes in gonadectomized fish and those in which laparotomy only was performed were the same as in normally maturing gonads.

Rodman, Duane T.
1963. Anesthetizing and air-transporting young white sturgeons. Progressive Fish-Culturist, vol. 25, no. 2, p. 71-78.

MS-222, tertiary amyl alcohol, and reduced temperature were employed in air-transporting young sturgeon. Shipments following use of two drugs were not successful. Fish exposed to a 1:40,000 solution of MS-222 survived for 30 to 48 hours but later died. A temperature of 40° F. provided adequate cold sedation which could be maintained during transport for approximately 30 hours with dry ice. The fish were shipped successfully by this method.

Rothlin, E.
1932. M.S. 222 (lösliches Anaesthesin), ein Narkotikum für Kaltblüter. Schweizerische Medizinische Wochenschrift, vol. 62, no. 45, p. 1042-1043.

MS-222 is a third as toxic to cold-blooded animals as novocaine and a tenth as toxic as cocaine. MS-222 was more efficacious in comparison with novocaine, strovain, alypin, tutokain, panthesin, kokaine, barokain, and eukain. A frog was completely anesthetized within 5 to 7 minutes in solutions of 1:1,000 to 1:2,000 of MS-222. Narcosis lasted several hours, and when the animal was placed in fresh water it recovered in 30 to 60 minutes. Studies with homologs of MS-222--allyl, isopropyl, n-butyl ester--gave no better results than the ethyl ester.

Rotmann, Eckhard.
1931. Die Rolle des Ektoderms und Mesoderms bei der Formbildung der Kiemen und Extremitäten von Triton. 1. Operation in Gastrulastadium. W. Roux Archiv fur Entwicklungsmechanik, vol. 124, p. 747-794.

There were no side or after effects of MS-222 on salamanders which were anesthetized by concentrations of 1:3,000 for 1 hour. Repeated anesthetization was not harmful.

Ryder, R. A.
1960. Comparative tagging returns employing three different anesthetics. Canadian Fish Culturist, no. 26, p. 23-25.

Ether, urethane, and MS-222 were used to anesthetize Stizostedion v. vitreum for tagging. After 2 years, approximately twice as many fish tagged with the help of MS-222 had been recovered as those tagged with the help of either of the other anesthetics.

Sakano, Ei-ichi.
1961. Anaesthetizing experiments of chum salmon fry with tricaine methanesulfonate (M.S. 222). Scientific Reports of the Hokkaido Salmon Hatchery, No. 16, p. 103-106.

Sandoz, M.
1920. Recherches expérimentales sur les anesthésiques locaux. 1. Préparations et propriétés physiologiques de la tricaïne et de quelques-un de ses dérivés, Bull. Soc. Vaud. Sc. Nat., vol. 53, p. 263-302.

Sandoz Pharmaceuticals
(No date) The toxicity of MS-222 to fish and frogs. Sandoz Pharmaceuticals, Hanover, N.J. (Mimeo) 2 p.

The 30-minute LC_{50} of MS-222 for frogs was 1:160.

A 1:12,200 concentration produced 50-percent mortality of young trout in 15 minutes. The maximal tolerated concentration (LC_1) for trout was 1:15,900 and a 1:25,000 solution induced anesthesia in 99 percent of the individuals (EC_{99}) within 3 to 4 minutes. These data gave a therapeutic index for MS-222 of 1.57.

A 10-percent solution of MS-222 which was stored at room temperature showed no loss in activity after 3 days. After 10 days there was a reduction in activity of about 5 percent. No difference was noted between solutions protected or not protected against light.

Sandoz Pharmaceuticals
(No date) M.S. 222-Sandoz, the anesthetic of choice in work with cold-blooded animals. Sandoz Pharmaceuticals, Hanover, N.J., Technical Bulletin. 10 p.

Concentrations of 0.5 to 1.0 g./gal. anesthetic silver salmon, sockeye salmon, lake trout, brown trout, and largemouth and smallmouth bass within 2 to 4 minutes at 40° to 60° F. A dosage of 0.25 to 1.0 g./gal. is recommended for rainbow trout.

A concentration of 0.14 g./gal. is recommended for tranquilizing bait fish during transport. Levels up to 0.32 g./gal. are effective for various tropical species.

Sato, T.
1930. Beiträge zur Analyse de Wolff'schen Linsen regeneration. Wilh. Roux Archiv für Entwicklungs mechanik d. Oragnismen, vol. 122, p. 451.

Schiffman, R. H., and P. O. Fromm.
1959. Measurement of some physiological parameters in rainbow trout (Salmo gairdnerii). Canadian Journal of Zoology, vol. 37, p. 25-32.

Rainbow trout were anesthetized in 300 p.p.m. of MS-222, placed on their backs and their hearts exposed. Blood samples were collected by cardiac puncture. The samples were divided for measurements of hematocrit, hemoglobin, and erythrocyte count and size. In addition, weights of organs and body water and volumes of blood and plasma were determined.

Schoettger, Richard A., and Arnold M. Julin.
1966. Investigations in Fish Control: 13. Efficacy of MS-222 as an anesthetic on four salmonids. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 19.

Concentrations of MS-222 ranging from 80 to 135 p.p.m. effectively anesthetized rainbow,

brown, brook and lake trout within 3 minutes at 7° to 17° C. The fish were exposed safely to these concentrations for 4 to 12 minutes. Dosages of 50 to 60 p.p.m. induced anesthesia within 15 minutes, and 15 to 30 p.p.m. were effective for sedation. The effective concentrations and exposure times were inversely related to temperature; however, the efficacy of sedating concentrations declined with time at 17° C. The narcotic action of the drug was reversible, provided the fish were removed from the anesthetic prior to the cessation of respiratory activity.

The efficacy of MS-222 was not affected significantly by size of fish or pH of the solution. Anesthetic solutions with hardnesses of 10 p.p.m. were less effective than those containing 35 to 180 p.p.m. total hardness, but the fish treated in soft water recovered sooner.

The exposure tolerances of fish which were repeatedly anesthetized with MS-222 were slightly greater than those which were unexposed previously.

Trout sedated in closed systems at 12° C. reduced their rate of oxygen consumption by 30 percent; the rate was not depressed significantly in open systems.

Serfaty, A., R. Labat, and R. Quillier.
1959. Les réactions cardiaques chez la carpe (Cyprinus carpio) au cours d'une anesthésié prolongée. Hydrobiologia, vol. 13, p. 144-151.

Anesthetizing carp with 100 p.p.m. of MS-222 caused a primary and secondary tachycardia. The first was believed to be correlated with encephalic penetration of the drug, and the second with a reduction of vagal tonus and lack of oxygen. Eventually, auriculoventricular dissociation occurred which was attributed to damage of the intracardiac system.

Shelton, G., and D. J. Randall.
1962. The relationship between heart beat and respiration in teleost fish. Comparative Biochemistry and Physiology, vol. 7, p. 237-250.

The heart and respiratory rates of tench were increased by anesthetizing them in 80 to 200 p.p.m. of MS-222. After 12 minutes in a 200 p.p.m. solution, the fish stopped breathing and the heart rate fell to a level similar to that of unanesthetized individuals. The heart beat and breathing became absolutely synchronized in animals lightly anesthetized in MS-222. The direct effect of MS-222 on isolated hearts was to decrease the beat rate at 15 p.p.m. and decrease beat amplitude above 30 to 60 p.p.m. Since fish have no sympathetic innervation of the heart, the authors suggested the presence of some cardioaccelerator fibers in the vagus nerve.

Smith, Lloyd L., Jr., Robert H. Kramer, and J. Cameron MacLeod.
1965. Effects of pulpwood fibers on fathead minnows and walleye fingerlings. Journal, Water Pollution Control Federation, vol. 37, no. 1, p. 130-140.

A concentration of 1,000 p.p.m. of MS-222 rapidly anesthetized fathead minnows for the measurement of hematocrits. The hematocrits of treated and control fish were not significantly different. The quantity of blood obtained from anesthetized individuals was 17 percent less than controls and probably indicates a reduced rate of circulation.

Smith, Lynwood S., and Gordon R. Bell.
1964. A technique for prolonged blood sampling in free-swimming salmon. Journal of the Fisheries Research Board of Canada, vol. 21, no. 4, p. 711-717.

The dorsal aortas of pink and sockeye salmon were cannulated to permit the sampling of blood over extended periods. The fish were anesthetized during the operation by irrigating the gills in a 1:15,000 solution of MS-222 using a pump recycling system. The cannula was attached to a length of polyethylene tubing which extended dorsal from the roof of the mouth to above the snout.

Snieszko, S. F.
1960. Microhematocrit as a tool in fishery research and management. U.S. Fish and Wildlife Service, Special Scientific Report--Fisheries No. 341. 15 p.

A technique for measuring the hematocrit of fish was described. The fish were anesthetized for approximately 1 minute in a 1:2,000 solution of MS-222.

Steinbrecht, Karl.
1957. Narkose von Fischen. Die Aquarien- und Terrarien-Zeitschrfit, vol. 10, no. 11, p. 305-306.

There were no abnormalities of offspring from guppies which were frequently anesthetized in a 1:2,000 solution of MS-222 following fertilization.

Steucke, Erwin, W., Jr., and Charles R. Atherton.
1965. Use of microhematocrit values to sex largemouth bass. Progressive Fish-Culturist, vol. 27, no. 2, p. 87-90.

MS-222 at 1:3,000 was used to anesthetize largemouth bass. They were narcotized in about 2 minutes.

Thompson, R. B.
1959. Tricaine methanesulfonate (M.S. 222) in transport of cutthroat trout. Progressive Fish-Culturist, vol. 21, no. 2, p. 96.

Young cutthroat trout were transported successfully in plastic bags which contained oxygen and a 1:40,000 solution of MS-222. The temperature was reduced by packing the bags in ice.

One-quart, plastic food boxes were tested in place of plastic bags. Approximately 500 individuals were added to each box which was half filled with the 1:40,000 solution of anesthetic. Oxygen was not used in these tests, but the containers were packed in ice. The fish were released after 3 hours, and only 19 out of 2,000 failed to recover from anesthesia.

Villwock, W.
1958. Narkose bei Fischen. Aquarien-Terrarien-Zeitschrift, vol. 11, p. 28.

Walker, Charles R., and Richard A. Schoettger.
1966. Investigations in Fish Control: 15. Residues of MS-222 in four salmonids following anesthesia. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 21.

Residues of MS-222 were measured in rainbow, brown, brook, and lake trout. The fish were anesthetized to medullary collapse in concentrations of 80 to 135 p.p.m., depending on species and temperature. Residues ranged from 6 to 72 p.p.m. in the muscle tissues of fish at the time of medullary collapse. The levels in fish which had recovered in freshwater at 12° C. declined rapidly within 3 hours and approached background values after 6 to 9 hours. A slower dissipation of residues occurred in tests at 7° and 17° C. The total residue including background did not exceed 5 p.p.m. at the end of 9 hours, or 3 p.p.m. after 24 hours for all species at all temperatures.

Residues of MS-222 in the blood, liver, and kidney of rainbow trout declined in a pattern similar to that for muscle.

1966. Investigations in Fish Control: 14. Method for determining MS-222 residues in fish. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 20.

The Bratton-Marshall method is sensitive for primary aromatic amines such as sulfa drugs. It was modified to detect MS-222.

Selected tissues from anesthetized trout were processed to extract the drug. The recovery of known amounts in muscle, blood, kidney, and liver ranged from 90 to 112 percent. The concentrations of interferring amines were evaluated.

The acute oral LD_{50} of MS-222 to laboratory rats is between 5 and 10 g./k. This indicates a relatively low toxicity of MS-222 to mammals.

Watson, John E.
1961. Tricaine methanesulfonate as an anesthetic for herring. Progressive Fish-Culturist, vol. 23, no. 4, p. 174.

MS-222 was tested in sea water as an anesthetic for herring. The fish were anesthetized within 8 minutes by a solution of 1:20,000 at 8° C. They recovered in approximately 8 minutes after removal to fresh seawater. Their prolonged exposure to MS-222 for 3 to 4 minutes after complete anesthesia was usually lethal.

Webb, Robert T.
1954. Tricaine methanesulfonate (M.S. 222) as an anesthetic for some common pond fishes. Unpublished thesis. Alabama Polytechnic Institute, Auburn.

Webb, Robert T.
1958. Distribution of bluegill treated with tricaine methanesulfonate (M.S. 222). Progressive Fish-Culturist, vol. 20, no. 2, p. 69-72.

Tests were conducted to determine whether the application of MS-222 would increase the number or pounds of bluegills which could be carried in a distribution truck. A concentration of 0.1 g./gal. appeared to be the most promising. The results of the tests were inconclusive; successful tests could not be duplicated; and at times the control fish hauled as well or better than the drugged ones.

Witschi, E.
1927. Testis grafting in tadpoles of Rana temporaria L. and its bearing on the hormone theory of sex determination. Journal of Experimental Zoology, vol. 47, p. 269.

Wood, E. M.
1956. Urethane as a carcinogen. Progressive Fish-Culturist, vol. 18, no. 3, p. 135-136.

Urethane induces lung tumors in mice which develop whether the drug is administered by injection, in drinking water, by nasal instillation, or from painting the skin.

An editorial comment accompanying this report indicated that MS-222 might be a suitable substitute for urethane.

17. MS-222 as an Anesthetic for Channel Catfish: Its Toxicity, Efficacy, and Muscle Residues

By Richard A. Schoettger, Charles R. Walker,
Leif L. Marking, and Arnold M. Julin

United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
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MS-222 AS AN ANESTHETIC FOR CHANNEL CATFISH: ITS TOXICITY, EFFICACY, AND MUSCLE RESIDUES

By Richard A. Schoettger, Fishery Biologist
Charles R. Walker and Leif L. Marking, Chemists
and Arnold M. Julin, Fishery Biologist
Bureau of Sport Fisheries and Wildlife
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Abstract.--MS-222 was tested as an anesthetic on channel catfish. Its acute toxicity is approximately 65 to 50 p.p.m. over periods of 24 to 96 hours. Anesthesia is induced within 2 minutes by concentrations above 100 to 140 p.p.m., and within 15 minutes by 70 p.p.m. Concentrations of 20 to 40 p.p.m. maintain sedation for 6 hours. Residues of MS-222 occur in muscles of anesthetized catfish, but decrease about 90 to 95 percent at 1 hour of withdrawal from the drug. Nine to 24 hours after withdrawal the residues decline to within the statistical variations of the background aromatic amines. The influences of duration of exposure, size of fish, temperature, and water quality on toxicity, efficacy, and residues are discussed.

MS-222 (tricaine methanesulfonate) is commonly employed to anesthetize fish during marking, spawning, and transporting. The drug has been used less commonly on channel catfish than on salmonids or centrarchids. In recent years the channel catfish, Ictalurus punctatus, has become highly regarded in many areas of the United States as a commercial and sport fish; it is being reared on rice-fish farms, in commercial hatcheries, and in farm ponds. We anticipate that a greater use of MS-222 will accompany increased effort to advance the culture and management of this species.

The U.S. Food and Drug Administration requires that certain disinfectants, antimicrobials, and anesthetics be cleared for their continued use on fish which may be consumed by people.

The information necessary for clearance of MS-222 as an anesthetic for channel catfish includes its toxicity, its efficacy as an anesthetic, and its residues in tissues of treated fish. Similar studies were conducted on salmonids at the Fish Control Laboratories (Marking, 1966; Schoettger and Julin, 1966; and Walker and Schoettger, 1966a, 1966b). The history and development of M-222 as a fish anesthetic were reviewed by Bové (1962), the manufacturer (Sandoz Pharmaceuticals), Eisler and Backiel (1960), and Schoettger (1966).

The scope of the investigation on channel catfish was somewhat narrower than that of our earlier study on salmonids. We tested the influences of temperature or size of fish on toxicity and efficacy. The effects of water hardness on toxicity and of pH on efficacy were also measured. Determinations of MS-222 residues in catfish were confined to those in muscle because residues in blood, liver, and kidney of salmonids decreased with withdrawal time at a rate similar to that in muscle; because background substances of natural origin in the livers and kidneys of

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salmonids interfered with analyses of low residues; and because muscle is the principal edible tissue in catfish.

METHODS AND MATERIALS

The channel catfish used in the toxicity, efficacy, and residue experiments were obtained from the national fish hatcheries at Fairport and Guttenberg, Iowa, and from the Mississippi River (by the Iowa Conservation Department). They were held in well water at 12° C. and without feed for 2 days before placement in the anesthetic solutions. Twenty-four hours before an experiment, the fish were acclimated to temperature in reconstituted water prepared according to methods described by Lennon and Walker (1964).

TOXICITY

The static bioassays of MS-222 were conducted with 1.9-, 2.6-, and 3.5-inch fish according to the methods of Lennon and Walker (1964). They were tested in 5-gallon glass jars containing 15 liters of test solution, with aeration applied to solutions containing the 3.5-inch fish. Water temperatures were maintained at 12°, 17°, and 22° C. by placing the bioassay vessels in thermostatically controlled water baths.

Ten fish were exposed to each concentration, and 10 or 20 served as controls. Ten concentrations were selected to yield survival and mortality of 1.9- and 2.6-inch fish; 5 concentrations were selected for the 3.5-inch fish.

The data were analyzed statistically according to the method of Litchfield and Wilcoxon (1949) to define concentrations which produced 50 percent mortality (LC_{50}). In addition, the variances, slope functions, and 95 percent-confidence intervals were determined.

Different water qualities were obtained by varying the amounts of reconstituting salts added to deionized water (table 1).

Safety indexes (S.I.) were determined for the anesthesia of channel catfish with MS-222. We define the indexes as the margin between concentrations effective for anesthesia and those which cause mortality, expressed as a number obtained by dividing the lethal concentration (LC_{50}) by the effective concentration (EC_{50}). An effective level anesthetizes the catfish to loss of equilibrium. This stage of anesthesia is defined later in methods for measuring the efficacy of MS-222.

The S.I. values were calculated for 15-, 30-, and 60-minute exposures. These exposures were selected to give consistent results because shorter exposures required extremely high concentrations. In longer exposures, the anesthetized fish occasionally recovered in the test solutions.

The maximum safety indexes (M.S.I.) were calculated from the LC_1 and EC_{99} values obtained by extrapolating the regressions used in determining LC_{50} and EC_{50} values. The maximum safety index is lower than the safety index and is biased in favor of greater safety.

Table 1.--Water qualities obtained with various amounts of reconstituting salts in deionized water

Water quality	Salts in mg./l.				Total hardness as $CaCO_3$ (p.p.m.)	Total alkalinity as $CaCO_3$ (p.p.m.)	pH
	$NaHCO_3$	$CaSO_4$	$MgSO_4$	KCl			
Soft	12	7.5	7.5	0.75	10-13	10-15	6.4-6.8
Standard	48	30.0	30.0	3.00	40-48	30-35	7.2-7.6
Hard	192	120.0	120.0	12.00	160-180	110-120	7.6-8.0

EFFICACY

Channel catfish of 2 to 6 inches and 7 to 12 inches were used to determine the efficacy of MS-222. Fish less than 4 inches were tested in 15 liters of reconstituted water. Larger individuals were exposed in 45 liters. Since preliminary trials failed to show that variable loading had any effect on efficacy, the loading levels were approximately 10 g./l. for the smaller fish, and up to 40 g./l. for the larger fish.

The methods of evaluating efficacy of MS-222 against channel catfish were similar to those reported in our trials with salmonids. A series of concentrations of MS-222 were tested at a temperature of 12° C. and at pH 7.0. The levels giving reasonable anesthetization, holding and recovery times, and which produced miminum mortality were considered for further trials at 7°, 12° 17°, 22°, and 27° C., and at pH 5.0, 7.0, and 8.5.

The criteria for assessing efficacy of MS-222 against channel catfish were changed slightly from those used for salmonids; therefore, a portion of the results which governed methodology are included in this section. The behavioral responses of catfish in deep anesthesia differed from those of trout. In catfish, at high concentrations of MS-222, we were unable to distinguish clearly the transition from total loss of equilibrium, stage II, into loss of reflex. The reflex response to constant pressure on the caudal fin or peduncle is frequently delayed for 1 to 10 seconds. Also, loss of reflex is not always distinct from medullary collapse. Opercular movements occasionally cease before reflex activity stops. A concentration of MS-222 was considered effective for rapid or moderately rapid anesthesia when it induced total loss of equilibrium, stage II, within 2 and 15 minutes respectively. This stage of anesthesia is more safely induced and maintained than loss of reflex, and the fish appear to be almost as easily handled. An effective concentration for sedation induces the response within 15 minutes and maintains it for 6 hours.

RESIDUES

Residues of MS-222 were measured in muscles of 6- to 13-inch channel catfish which had been treated during the efficacy trials. Groups, each composed of three individuals, were anesthetized to medullary collapse by 270 p.p.m. of MS-222 at 7°, 12°, 17°, 22°, and 27° C. In this series of tests, the drug was less effective at 7° and some fish tolerated an exposure exceeding 1 hour; at higher temperatures the exposures were approximately 5 to 10 minutes long.

Five or six groups of catfish were anesthetized at each temperature. One narcotized group was selected for each temperature for analyses of residues at 0-hour withdrawal. The remaining groups were placed in fresh water for recovery. Their muscles were analyzed 1, 3, 6, 9, and 24 hours after withdrawal. We also analyzed control fish at each temperature.

The residues of MS-222 in fish flesh were determined by the modified Bratton-Marshall method developed by Walker and Schoettger (1966a).

RESULTS

TOXICITY

Effects of size.--The toxicity of MS-222 to channel catfish is dependent, in part, on their size. The larger fish appear more resistant to the anesthetic than smaller individuals (table 2). The 24-hour LC_{50} values for 1.9-, 2.6-, and 3.5-inch specimens are 58.0, 64.0, and 66.2 p.p.m., respectively. This size-toxicity relationship was also observed in the 48- and 96-hour bioassays.

Effects of temperature.--The toxicity of MS-222 to channel catfish is influenced by temperature. The data indicate a higher resistance of 1.9-inch fish at 17° than at 12° or 22° C. (table 2). The 24-hour LC_{50} values for the latter two temperatures are similar, but

Table 2.--Toxicity of MS-222 to channel catfish at three temperatures

Temperature and lot	Average size		24 hours		48 hours		96 hours	
	Length (in.)	Weight (g.)	LC_{50} (p.p.m.)	95-percent confidence interval	LC_{50} (p.p.m.)	95-percent confidence interval	LC_{50} (p.p.m.)	95-percent confidence interval
At 12° C.:								
Lot 276.....	1.9	0.9	58.0	55.6-61.5	55.0	53.3-56.8	51.1	49.0-53.5
Lot 92.....	2.6	1.9	64.0	58.2-70.4	55.0	52.9-57.2	55.0	53.9-56.1
Lot 122.....	3.5	5.2	66.2	63.0-69.5	62.1	60.9-63.3	62.1	60.9-63.3
At 17° C.:								
Lot 276.....	1.9	0.9	60.5	58.1-62.9	60.0	58.3-61.7	60.0	58.3-61.7
At 22° C.:								
Lot 276.....	1.9	0.9	59.8	58.0-61.5	58.8	57.0-60.6	58.8	57.0-60.6

Table 3.--Toxicity of MS-222 in selected water qualities at 12° C.

Total hardness	Total alkalinity (p.p.m.)	24 hours			48 hours		96 hours	
		pH	LC_{50} (p.p.m.)	95-percent confidence interval	LC_{50} (p.p.m.)	95-percent confidence interval	LC_{50} (p.p.m.)	95-percent confidence interval
12 p.p.m...	15	6.6	64.0	58.7-69.8	60.0	57.1-63.0	50.0	46.7-53.5
46 p.p.m...	30	7.5	58.0	55.6-61.5	55.0	53.3-56.8	51.1	49.0-53.5
170 p.p.m..	114	7.8	54.0	51.0-57.4	54.0	51.0-57.4	54.0	52.6-55.5

the 96-hour LC_{50} was lower at 12° than at 22° C. In general, the relative change in toxicity of the anesthetic with exposure is less at 17° and 22° than at 12° C.

Effect of water quality.--The toxicity of MS-222 to catfish was evaluated in relatively soft, medium, and hard water (table 3). The LC_{50}'s range from 50 to 64 p.p.m., and the drug is more toxic at 24 hours in hard water than in soft water. After 96 hours of exposure the drug appears more toxic in soft water. The toxicities of MS-222 to catfish in medium and hard waters are similar, except at 96 hours of exposure. Here, the LC_{50} values for medium and soft water are much alike, and the 95-percent confidence limits overlap considerably.

The greatest change in toxicity of MS-222 with exposure occurred in tests in soft water. These trials also gave the most variable results as shown by the relative widths of the 95-percent confidence intervals (table 3).

Safety indexes.--The S.I. values in table 4 range from 2.4 to 3.0 and indicate that 15-minute exposures are safer than those lasting 30 or 60 minutes. The M.S.I. values, on the other hand, show greater safety with longer exposures. This is probably due to the large variance which is associated with the broad range of concentrations tolerated by channel catfish.

EFFICACY

The efficacy of MS-222 for inducing rapid and moderately rapid anesthesia and sedation in channel catfish appears to be unaltered by pH 5.0 to 8.5. These data were pooled, without differentiation, with those for size of fish and temperature.

Rapid anesthesia.--Concentrations of 140 to 270 p.p.m. are all equally effective for the rapid anesthesia of 7- to 12-inch catfish at 12° to 27° C. (table 5). Levels above 100 to 120 p.p.m. anesthetize 2- to 6-inch fish.

Table 4.--Safety indexes and maximum safety indexes for anethesia of channel catfish with MS-222 at 12° C.

Exposure	Safety index			Maximum safety index		
	LC_{50} (p.p.m.)	EC_{50} (p.p.m.)	Index LC_{50}/EC_{50}	LC_1 (p.p.m.)	EC_{99} (p.p.m.)	Index LC_1/EC_{99}
15 minutes.....	139.0	46.5	3.0	76.0	71.9	1.1
30 minutes.....	118.0	45.0	2.6	74.0	62.0	1.2
60 minutes.....	110.0	46.4	2.4	80.0	62.8	1.3

Table 5.--Concentrations of MS-222 producing rapid anesthesia in two sizes of channel catfish at five temperatures

Temperature and concentration	Size of fish (in.)	Lot No.	Fish in loss of equilibrium, stage II, within 2 minutes		Mean range of exposure times (min.)		Safe exposure index[1]	Recovery mean time range (min.)	Survival (percent)
			Number	Percent	First fish	Last fish			
At 7° C.:									
160-270 p.p.m.	2- 6	122	39/ 40	98	[2] 6	--	--	6-24	100
160-270 p.p.m.	7-12	114	24/ 30	80	[2] 6	--	--	6-13	100
200-270 p.p.m.	7-12	199 & 203	2/ 48	4	25	48	--	19-33	98
At 12° C.:									
80 p.p.m.	2- 6	122	0/ 40	0	[2] 30	--	--	2- 5	100
100 p.p.m.	2- 6	122	10/ 20	50	[2] 11	--	--	2- 9	100
120-260 p.p.m.	2- 6	122	331/345	95	[2] 7	--	--	7-18	97
270 p.p.m.	7-12	203	18/ 18	100	6	7	3.0	7-10	100
At 17° C.:									
80 p.p.m.	2- 6	122	0/ 10	0	[2] 20	--	--	8-15	100
100-220 p.p.m.	2- 6	122	90/ 90	100	[2] 6	--	--	3- 7	100
140-270 p.p.m.	7-12	114 & 203	63/ 80	79	8	11	4.0	4- 8	100
At 22° C.:									
270 p.p.m.	7-12	203	24/ 24	100	4	5	2.0	5- 7	100
At 27° C.:									
100-130 p.p.m.	7-12	203	12/ 25	48	7	9	--	3- 9	100
140-270 p.p.m.	7-12	203	53/ 55	96	4	5	2.0	5-10	80

[1] Index obtained by dividing the time for the first fish to reach medullary collapse by the time (2 min.) for all fish to reach loss of equilibrium, stage 11.

[2] Fish removed from the anesthetic before all reach medullary collapse.

Catfish to tolerate exposures to MS-222 for approximately 6 to 11 minutes at 12° and 17°, and 4 to 5 minutes at 22° and 27° C. They usually recover in fresh water in less than 15 minutes; at 27°, however, 20 percent of the test fish died when they were not removed quickly enough to prevent overexposures.

The results of tests at 7° C. were variable (table 5). Concentrations of 160 to 270 p.p.m. were 80 to 98 percent effective on lots 114 and 122, but 200 to 270 p.p.m. were ineffective on lots 199 and 203. Although individuals in lots 114 and 122 were effectively anesthetized and tolerated exposure for about 6

minutes, lots 199 and 203 required 3 to 5 minutes for anesthesia, and some individuals tolerated exposures for more than an hour.

Fish in lot 199 were used for residue analyses, and the numbers remaining were insufficient for comparative tests to determine whether they were also more resistant than lot 114 at higher temperatures. The sensitivities of catfish in lot 203 were similar to those in lot 199 at 7° and those in lot 114 at 17° C., and the results were combined at the respective temperatures. The purpose in comparing sensitivities of the different lots is to point out that there was some variable in the tests at 7° which increased the resistance of catfish to MS-222 in one instance but not in another. Analysis of the sources of the fish and their general conditions indicated that these were probably not causes of the inconsistencies. On the other hand, fish in lot 114 were tested in the fall, and those in lots 199 and 203 in the spring. It is conveivable that the natural acclimation of the latter fish to low temperatures during the winter may have contributed to their resistance to MS-222 at 7° C.

We were unable to calculate safe exposure indexes for all of the trials reported in table 5. In most instances the fish were placed in fresh water before they all entered medullary collapse. The indexes in the remaining trials range from 2 to 4. They indicate that catfish can be safely exposed to MS-222 for about 2 to 4 times longer than required to induce loss of equilibrium, stage II.

Moderately rapid anesthesia.--Seventy p.p.m. of MS-222 induce loss of equilibrium in channel catfish within 15 minutes (table 6). They tolerate exposure to this concentration

Table 6.--Concentrations of MS-222 producing moderately rapid anesthesia in two sizes of channel catfish at four temperatures

Concentration, temperature and size of fish	Lot No.	Fish in loss of equilibrium, stage II, within 15 minutes		Exposure time (min.)	Recovery	
		Number	Percent		Mean time range (min.)	Survival (percent)
At 70 p.p.m.:						
At 7° C.:						
2- 6 in.	122	10/10	100	30	2- 4	100
7-12 in.	114	6/ 6	100	30	2- 6	100
At 12° C.:						
2- 6 in.	122	70/70	100	30	3- 5	100
7-12 in.	114	33/35	94	30	2-10	100
At 17° C.:						
2- 6 in.	122	10/10	100	30	1- 2	100
At 60 p.p.m.:						
At [illegible] C.:						
[illegible]-12 in.	203	4/10	40	[1] 24	--	100
At 70 p.p.m.:						
At 27° C.:						
7-12 in.	203	8/8	100	180	1- 2	75

[1] Hours

for at least 30 minutes and many can be exposed longer. In one test, at 27° C., the majority of the fish survived at 3-hour exposure.

Water temperature and size of fish appear to have no influence on the efficacy of a concentration inducing a moderate rate of anesthesia. The fish recover faster, however, at 22° and 27° than at 7°, 12°, or 17°. The sensitivities of the different lots of test fish at various temperatures were only partially compared.

A moderate rate of anesthesia appears most applicable to handling operations such as measuring and weighing, or when large numbers of individuals are involved. A level of 70 p.p.m. may also be useful for long-term surgical operations, provided sufficient time is allowed for reflex responses to decline.

Sedation.--Channel catfish are sedated in 20 p.p.m. of MS-222 at 7°, 12°, and 17° C. (table 7). This level was not effective at 27° C., and the concentration was increased to 40 p.p.m. to maintain sedation for 6 hours. These data suggest that sedating concentrations of MS-222 are metabolized or deactivated at higher temperatures. This finding is supported by the results of McFarland (1959 and 1960), Schoettger and Julin (1966), and our toxicity trials with catfish.

RESIDUES

Residues of MS-222 occur in the muscles of anesthetized channel catfish and appear to vary with temperature. Seventeen to 147.2 p.p.m. of the drug, including background aromatic amines, were measured in fish narcotized to medullary collapse in 270 p.p.m. of MS-222 (table 8). The mean concentrations of residue at 0-hour withdrawal are: 31.3 p.p.m. at 7° C., 69.9 p.p.m. at 12°, 125.5 p.p.m. at 17°, 97.5 p.p.m. at 22°, and 65.7 p.p.m. at 27°. The levels in fish treated at 12°, 17°, 22°, and 27° are two to four times greater than in those exposed at 7° C.

Table 7.--Concentrations of MS-222 producing sedation in two sizes of channel catfish at four temperatures

Concentration, temperature, and size of fish	Lot No.	Fish in sedation at--				Behavior[1] of fish not in sedation at--	
		15 minutes		6 hours			
		Number	Percent	Number	Percent	15 min.	6 hrs.
At 20 p.p.m:							
At 7°C.:							
2-6 in.	122	10/ 10	100	10/ 10	100	--	--
7-12 in.	114	6/ 6	100	6/ 6	100	--	--
At 12° C.:							
2- 6 in.	122	138/140	99	132/140	94	< >	>
At 17°C:							
2- 6 in.	122	50/ 50	100	50/ 50	100	--	--
7-12 in.	114	6/ 6	100	6/ 6	100	--	--
At 27°C.:							
7-12 in.	203	12/ 12	100	0/ 12	0	--	<
At 30 p.p.m:							
At 27°C.:							
7-12 in.	203	13/ 13	100	8/ 13	62	--	<
At 40 p.p.m.:							
At 27°C.:							
7-12 in.	203	6/ 6	100	[2] 6/ 6	100	--	--

[1] > = deeper anesthesia; < = similar to controls.
[2] Fish in sedation at 3 and 19 hours.

Table 8.--Residues of MS-222 including background in muscle of channel catfish for various withdrawal times at selected temperatures

Temperature and withdrawal time	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (in.)	Weight (g.)	Mean	Range		
At 7° C.:							
0 hour	3	12.13	199.7	31.30	17.0- 38.5	7.26	---
1 hour	3	12.53	223.0	26.70	22.5- 34.9	4.10	8.58-44.34
6 hours	3	12.53	214.3	8.83	7.6- 11.2	1.18	1.08-16.58
9 hours	3	12.03	223.3	5.07	4.0- 5.8	0.55	2.70- 7.44
24 hours	3	12.77	248.0	4.53	2.5- 7.6	0.91	0.61- 8.45
At 12° C.:							
0 hour	3	10.57	140.7	69.93	56.8- 77.2	6.58	---
1 hour	3	7.87	47.3	6.57	4.9- 9.3	1.40	0.55-12.59
3 hours	3	8.27	61.7	1.20	0.5- 1.2	0.47	0.00- 3.22
6 hours	3	7.73	45.0	1.10	0.8- 1.3	0.15	0.45- 1.75
9 hours	3	8.07	51.7	0.83	0.6- 1.0	0.18	0.06- 1.60
24 hours	3	7.87	49.3	0.90	0.8- 1.0	0.06	0.64- 1.16
At 17° C.:							
0 hour	3	9.00	77.0	125.53	88.6-147.2	18.55	---
1 hour	3	8.83	67.7	6.57	4.8- 8.7	1.15	1.62-11.52
3 hours	3	9.20	80.7	1.40	1.3- 1.5	0.05	1.19- 1.62
9 hours	3	9.33	84.3	2.23	1.7- 2.8	0.32	0.85- 3.61
24 hours	3	9.00	78.3	1.00	0.7- 1.4	0.21	0.10- 1.90
At 22° C.:							
0 hour	3	8.90	67.3	97.47	87.6-105.4	5.23	---
1 hour	3	7.70	45.7	6.57	4.5- 9.3	1.43	0.42-12.72
3 hours	3	8.83	65.3	1.47	1.2- 1.9	0.22	0.52- 2.42
6 hours	3	7.53	41.0	1.20	0.9- 1.7	0.25	0.12- 2.28
9 hours	3	8.17	56.7	1.27	0.8- 1.8	0.29	0.02- 2.52
24 hours	3	8.47	57.3	0.60	0.5- 0.7	0.06	0.34- 0.86
At 27° C.:							
0 hour	3	8.80	71.7	65.67	37.2- 80.0	14.23	---
1 hour	3	9.13	71.0	4.00	2.0- 6.4	1.29	0.00- 9.55
3 hours	3	9.23	80.0	4.23	1.9- 6.8	0.59	1.67- 6.77
6 hours	3	8.83	64.3	1.47	0.9- 1.9	0.30	0.18- 2.76
9 hours	3	8.83	64.0	1.27	0.8- 1.6	0.24	0.24- 2.30
24 hours	3	8.77	62.0	0.77	0.6- 1.0	0.12	0.25- 1.29

As in salmonids, a background of aromatic amines is also present in the muscles of unanesthetized catfish. The background in catfish ranges from 0.5 to 3.6 p.p.m. (table 9). Both the upper and the lower limits of the range occur in samples from fish (lot 199, table 5) maintained at 7° C. The mean background at this temperature is 1.7 p.p.m. with a standard error of 0.96. The 95-percent confidence interval around the means is 0 to 5.33 p.p.m. The mean concentrations in flesh at other temperatures range from 0.8 to 1.5 p.p.m. with standard errors from 0.05 to 0.18. Their 95-percent confidence intervals overlap between 0.10 and 1.72 p.p.m.

The residues of MS-222 in muscles of catfish decline approximately 90 to 95 percent after 1 hour of withdrawal at 12°, 17°, 22°, and 27° C. (table 8). They decrease further after 6 to 24 hours of withdrawal and are withdrawal and are within the 95-percent confidence intervals around the mean backgrounds of controls. The residues in catfish at 7° decline more slowly than at higher temperatures, but are within the background

Table 9.--Background residues of aromatic amines in muscle of channel catfish used as controls in the study of MS-222

Temperature	Number of fish	Mean size		Concentration in p.p.m.		Standard error	95-percent confidence interval
		Length (in.)	Weight (g.)	Mean	Range		
7° C.....	3	12.20	220.7	1.70	0.5-3.6	0.96	0.00-5.83
12° C.....	5	9.80	108.2	0.80	0.6-1.0	0.07	0.61-0.99
17° C.....	3	9.20	80.7	1.07	0.9-1.3	0.12	0.55-1.59
22° C.....	3	9.43	80.7	1.50	1.4-1.5	0.05	1.28-1.72
27° C.....	3	8.37	51.7	0.87	0.6-1.2	0.18	0.10-1.65

Table 10.--Acetylated aromatic amines in muscle of channel catfish for various withdrawal times at 7° C.

Withdrawal time	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval	Percent of total aromatic amines
		Length (in.)	Weight (g.)	Mean	Range			
0 hour.....	3	12.1	199.7	1.07	0.0-2.4	0.71	---	2.8
1 hour.....	3	12.5	223.0	3.76	2.4-5.1	0.78	0.40-7.12	12.4
6 hours.....	3	12.5	214.3	5.50	4.8-6.2	0.40	3.76-7.24	38.4
9 hours.....	3	12.0	223.3	6.00	5.3-7.0	0.56	3.61-8.29	54.2
24 hours.....	3	12.8	248.0	3.83	2.4-4.9	0.75	0.62-7.04	47.9

confidence interval for this temperature after 9 to 24 hours of withdrawal.

Channel catfish which had been anethetized at 7° were also analyzed to detect possible acetylated derivatives of MS-222 (table 10). The presence of these derivatives may indicate a mechanism within the fish for deactivation of the drug. The acetylated fraction is obtained by subtracting the free MS-222 and background concentrations (table 8) from the total aromatic amines measured in the sample after acid hydrolysis. At 0-hour withdrawal the muscles contain an average of 1.07 p.p.m. of acetylated aromatic amines, or 2.8 percent of the total aromatic amines. When the fish are transferred to fresh water, the concentrations of acetylated substances increase to a mean high of 6 p.p.m. after 9 hours of withdrawal, and then decline. Within this period, free residues decrease so that the relative amount of acetylated amines increase to 54.2 percent. The concentrations of both fractions decline at 24 hours of withdrawal, but their proportions are similar.

DISCUSSION

TOXICITY

The channel catfish is one of the species more resistant to MS-222. The 24- to 96-hour LC_{50}'s range from 66.2 to 51.1 p.p.m. Marking (1966b) reported LC_{50}'s for similar exposures of 52.2 to 31.0 p.p.m. for various salmonids and 61.5 to 39.4 p.p.m. for northern pike, bluegill, largemouth bass, and walleye. This greater resistance of catfish to certain chemicals has been noted in other investigations at this laboratory (Walker et al., 1964; Marking, 1966a). Also, large catfish are more resistant to the drug than small ones, and this finding agrees with the size-sensitivity relation between other species and MS-222 noted by Marking (1966b).

Catfish are somewhat more resistant to MS-222 at 17° than at 12° or 22° C. Marking (1966b) made a similar observation on the toxicity of the anesthetic to bluegill. Whether the resistance of 1.9-inch catfish at 17° C. is

significant seems doubtful. The confidence interval for the 24-hour LC_{50} at this temperature includes the LC_{50} for 1.9-inch fish at 22° and almost includes the value at 12°.

The interaction of temperature and exposure seems to have a greater effect on toxicity of MS-222 than temperature alone. The fact that the drug increases in toxicity with time at 12°, but not significantly at 17° and 22° suggests that it degrades at the higher temperatures.

We found that catfish are relatively more resistant when the anesthetic is dissolved in soft water. The resistance declines with exposure, and after 96 hours the soft water solutions are slightly more toxic. Schoettger and Julin (1966) reported that MS-222 is less effective against rainbow trout in very soft water. They suggested that the rate of absorption and deactivation of the drug may increase along with higher metabolic rates of trout in soft water. The decreased resistance of catfish with time, however, may reflect the effects of osmotic stress.

The safety indexes for anesthesia of channel catfish with MS-222 show that the lethal concentrations of drug are approximately 2.5 to 3.0 times greater than the levels which induce narcosis. In actual practice this method of evaluating safety of fish anesthetics is not entirely applicable. The fish may be anesthetized at similar or slightly higher concentrations than those used to calculate safety indexes, but exposure may determine lethality. For this reason some indexes based on safe exposure were calculated in the efficacy experiments.

EFFICACY

MS-222 is effective and safe for the rapid and moderately rapid anesthesia of channel catfish. The concentrations for the former rate of narcotization, 100 to 270 p.p.m., exceed the 80 to 135 p.p.m. which were effective for salmonids (Schoettger and Julin, 1966) and suggest that catfish tolerate a much broader range of concentrations. The levels for moderate rates in salmonids and catfish are similar. The low end of the range of concentrations inducing rapid anesthesia in catfish probably represents a threshold, or minimum effective level, since concentrations up to 270 p.p.m. are essentially no more effective. Concentrations within this range have been used to anesthetize channel catfish for sexing and physiological investigations (Crawford and Hulsey, 1963; Larsen, 1964; Moss and Scott, 1964).

The inconsistent efficacy of MS-222 on three lots of fish at 7° C. may reflect differential sensitivities of the lots. The differences may be related to prior acclimation to environmental temperatures. Residues of MS-22 were later measured in muscles of fish in a resistant lot and, as discussed elsewhere, the residues differ markedly from those in fish treated at higher temperatures.

In general, temperature has little influence on the efficacy of MS-222 against catfish, but they tolerate less exposure at 22° and 27° than at 7°, 12°, or 17° C. It was necessary to increase the concentrations of the drug for salmonids at lower temperatures, but a similar influence of temperature on the resistance of catfish may have been masked by the broad range of effective concentrations.

Although channel catfish appear to be more resistant to MS-222 than salmonids, the durations of exposure tolerated by both are similar. The behavioral responses of the catfish must be observed, however, to minimize the risk of overexposures. Medullary collapse occurs almost simultaneously with loss of reflex, and therefore, anesthesia to the latter stage may be unsafe and unnecessary. The delayed reflex response which occurs late in loss of equilibrium does not usually preclude the handling of fish. The cessation of opercular activity in catfish, as in trout, is a good criterion for transferring them to fresh water, although many individuals may recover completely from brief exposures beyond this point.

Sedation can be induced and maintained in channel catfish by MS-222 at 20 to 40 p.p.m., depending on temperature. Schoettger and Julin (1966) cited contradictory reports on

the benefits of sedating various species of fish with the drug for transport. We are unable to locate published reports on the use of MS-222 for transport of channel catfish.

RESIDUES

The mean residues of MS-222 in the flesh of anesthetized channel catfish at 12° and 17° C. are approximately two to eight times as great as mean residues measured by Walker and Schoettger (1966a) in salmonids at the same temperatures. The mean values at 7° are similar. This indicates that relatively more MS-222 is taken up by salmonids at colder temperatures than at warmer temperatures, whereas the reverse applied to catfish. One explanation for the differential deposition of MS-222 in salmonids and catfish may be the influence of temperature on the efficacy of MS-222 in salmonids, and a relative lack of its effect in catfish. The concentrations of MS-222 were adjusted upward to maintain efficacy against salmonids at lower temperatures, but only one level, two to three times that for salmonids, was used to anesthetize catfish in the residue experiments. On the other hand, the differential may represent the influence of theoretical temperature optimums or acclimation on drug metabolism.

Residues of MS-222 in salmonids and channel catfish decline rapidly during the first hour of withdrawal, except those in catfish at 7°. The latter are initially low and decrease gradually. The acclimation of these individuals to winter temperatures may have contributed to their low uptake and turnover of MS-222, and to their resistance to anesthetization at 7°. The background of aromatic amines in untreated catfish is slightly higher at 7° and suggests a temperature-related reduction in metabolism of these substances. Residues of the drug, at all temperatures, cannot be distinguished from the background in controls when the fish are held in fresh water for 9 to 24 hours after anesthesia.

Interestingly, the mean concentrations of residues at 0-hour withdrawal form a parabolic curve when plotted against temperature, and peak at 17° C. We have no explanation for this, unless it is caused by a temperature-dependent, uptake-and-metabolism relation. The pattern does not persist after withdrawal.

The acetylation of MS-222 may be an effective mechanism for its deactivation in catfish. The concentrations of acetylated derivatives in muscles of fish treated at 7° increase during withdrawal times up to 9 hours, and as the amount of free MS-222 decreases. They decline after 24 hours of withdrawal, when the concentration of the free fraction is lowest. The inverse change in concentrations of free and acetylated derivatives during withdrawal suggests metabolism of MS-222. The absolute amounts of acetylated substances are relatively small, however, and may simply reflect the turnover of natural aromatic amines.

SUMMARY

MS-222 was tested against channel catfish to determine its toxicity, efficacy, and residues in muscle. Several size groups of fish were exposed to solutions with various temperatures, pH, and water qualities.

The LC_{50} values of MS-222 for catfish range from 66.2 to 51.1 p.p.m. in 24- to 96-hour bioassays. The variations in toxicity are associated with exposure, size of fish, temperature, and water quality. Channel catfish are more resistant to the drug than other species we have tested. The safety indexes for anesthesia of catfish decrease from 3.0 to 2.4 with exposures of 15 to 60 minutes.

Concentrations of drug above 100 to 140 p.p.m. are effective for inducing rapid loss of equilibrium in catfish within 2 minutes. They tolerate exposure for 4 to 11 minutes. A level of 70 p.p.m. produces moderately rapid anesthesia, and 20 to 40 p.p.m. are effective for sedation. The fish tolerate concentrations for moderately rapid anesthesia for at least 30 minutes. They can be maintained in sedation for 6 hours by 20 to 40 p.p.m. The efficacy of MS-222 is not influenced greatly by temperature, pH, or size of fish. At 22° or 27° C., the fish tolerate less exposure to high drug concentrations, and at 27° the sedation concentration has to be increased to compensate for an apparent degradation of MS-222.

The deep stages of MS-222-induced narcosis, beyond loss of equilibrium, appear less distinct in catfish than in salmonids. The behavior of the fish must be observed to minimize the risk of overexposures, and cessation of opercular activity is a good criterion for transferring them to fresh water.

Residues of MS-222 occur in the muscles of anesthetized catfish. They vary, according to temperature, from 17.0 to 147.2 p.p.m. including background aromatic amines. The background, in control fish, ranges from 0.5 to 3.6 p.p.m. The lowest concentrations of residue at 0-hour withdrawal occur in fish treated at 7° C., but after 1 hour of withdrawal from the drug the levels in individuals exposed at this temperature are highest. The residues in muscles after 9 to 24 hours of withdrawal at all temperatures decline to within the statistical variations in the background concentrations.

The concentrations of acetylated aromatic amines in muscles of catfish anesthetized at 7° C. increase as MS-222 residues decrease during 9 hours of withdrawal. The inverse relation suggests metabolism of MS-222 by acetylation, but the small amounts of acetylated derivatives may be aromatic amines of natural origin.

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